## Hematopoietic stem cell transplantation in B- and T-cell lymphoma

Visser, O.

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# Radioimmunotherapy for Indolent B-Cell Non-Hodgkin Lymphoma in Relapsed, Refractory and Transformed Disease

*Otto J. Visser*,[1] *Lars R. Perk*,[2] *Josée M. Zijlstra*,[1] *Guus A.M.S. van Dongen*,[2] *Peter C. Huijgens*[1] and *Arjan A. van de Loosdrecht*[1]

1 Department of Hematology, VU University Medical Center, Amsterdam, The Netherlands
2 Department of Otolaryngology/Head and Neck Surgery, VU University Medical Center, Amsterdam, The Netherlands

## Abstract

Radioimmunotherapy (RIT) is a new treatment modality that combines the benefits of radiotherapy and immunotherapy. In RIT, a radionuclide is coupled to a monoclonal antibody, directed against an antigen expressed on tumor cells. Recently, RIT has been introduced targeting the CD20 surface antigen, which is expressed on nearly all B-cell non-Hodgkin lymphomas (NHL).

Clinical experience with RIT in the treatment of patients with indolent NHL is increasing. To date, two commercially available agents are used: yttrium-90 ($^{90}$Y)-ibritumomab tiuxetan and iodine-131 ($^{131}$I)-tositumomab. In general, there is no organ-specific non-hematologic toxicity when a standard dose of RIT is used. Bone marrow suppression is the dose-limiting RIT toxicity; therefore, bone marrow infiltration by NHL should be investigated before treatment. Treatment-related myelodysplastic syndromes and acute myeloid leukemia after RIT are being investigated but long-term data are needed for final evaluation.

Results are quite encouraging with respect to complete remission and overall response, even in pretreated patients with unconjugated monoclonal antibodies. RIT induces high response rates and a significant subgroup of patients has achieved long-term durable responses. RIT is feasible in heavily pretreated patients and does not compromise future treatments in the event of progressive disease. Randomized phase III studies are in progress to evaluate the timing of RIT in the overall management of indolent NHL

Investigations of new emerging therapeutic strategies for patients with indolent NHL are underway, with research into the feasibility of RIT as first-line therapy and in advanced disease, RIT dose escalation and combined modality approaches with autologous stem cell transplantation. The encouraging results of RIT in indolent NHL have initiated studies focusing on its benefit for patients with aggressive NHL.

B-cell non-Hodgkin lymphoma (NHL) comprises approximately 4% of all newly diagnosed cancers each year worldwide, and is the most commonly diagnosed hematologic malignancy. The annual incidence in the US and Europe is 15/100 000 persons.[1] Indolent B-cell NHL represents approximately 45% of all lymphomas. Of all indolent NHL, follicular B-cell NHL is by far the most common subtype in NHL.

Follicular NHL is characterized by an advanced stage at presentation and an indolent clinical course resulting in a long median survival (8–10 years).[2,3] If treatment is indicated, patients usually respond to therapy, but the clinical course is characterized by recurrent disease ultimately leading to death. Death may be due to refractory disease, transformation to aggressive NHL, infections, or complications of treatment. The prognosis after transformation is usually poor, with a median survival of <1 year.

For treatment of relapsed disease, there are many chemotherapeutic and immunological treatment options available, but to date, there is no significant impact on overall survival.[4] Recently, radioimmunotherapy (RIT) has been introduced, which uses radionuclides coupled to monoclonal antibodies (MAbs) to deliver radiation specifically to the sites of disease after targeting. Follicular B-cell NHL is a favorable target for RIT because it is common-

ly positive for the surface antigen CD20, to which MAbs are available for clinical use.

## 1. Basic Principles of Radioimmunotherapy (RIT)

RIT is an internal radiation treatment involving administration of radionuclide-conjugated antibodies that are directed against antigens expressed on tumor cells. The bloodstream carries the antibodies to the tumor where the radionuclides are able to affect malignant cells. Its advantage is specific tumor irradiation, with less damage to normal tissue than external beam radiation. Research in RIT is ongoing with respect to optimizing biodistribution, choice of tumor antigens, and radionuclides.

RIT has specific characteristics that make it effective in the treatment of NHL. Cell surface antigens such as CD20 are expressed on lymphoma cells, which can be exploited as target antigen. The CD20 antigen is a pan-B-cell antigen, and is homogenously expressed on almost all B-cell lymphomas at high density. CD20 is not expressed on hematological stem cells or differentiated cells such as plasma cells, nor on non-hematopoietic tissues. CD20 is also characterized by minimal modulation or internalization after MAb binding.

Unlabeled CD20 MAbs are widely used in the treatment of indolent and aggressive NHL, with or without chemotherapy.[5,6] These MAbs are capable of directly killing lymphoma cells by complement-mediated effects, antibody dependent cellular cytotoxicity, and a direct cytotoxic effect. MAbs directed against other antigens on lymphoma cells, such as CD19 and CD22, are under investigation.

One important characteristic of lymphoma cells is that they are very radiosensitive. In conventional external beam radiotherapy, γ-radiation is delivered at relatively high dose rates for short periods of time and separated by intervals without radiotherapy. In RIT, the peak dose rate is lower, but radiation is delivered continuously at an exponentially declining rate for several days as radionuclides decay. The continuous irradiation by RIT may prevent cellular DNA repair mechanisms in lymphoma cells. The administered dose is the therapeutic amount of radioactivity given to a patient, and is measured in mCi or MBq. The absorbed dose in tissues is measured in cGy by dosimetry, and can be used for prediction of anti-tumor effects and toxicity.

Studies of RIT in patients with refractory or relapsed disease after treatment with unmodified or 'cold' antibodies such as rituximab (Biogen Idec Inc. and Genentech Inc.) do show response to RIT.[7-10] This indicates an important role for the radionuclides attached to MAbs and their radiotherapeutic properties.[11-13] Commercially available agents used in CD20-positive NHL are yttrium-90 ($^{90}$Y)-ibritumomab tiuxetan (Zevalin®; Biogen Idec Inc. and Schering AG)[1] and iodine-131 ($^{131}$I) -tositumomab (Bexxar®; Corixa Corporation and GlaxoSmithKline). Both radiolabeled MAbs are of murine origin, and development of human-anti-mouse-antibodies (HAMAs) has been demonstrated. $^{90}$Y is conjugated to ibritumomab by the chelator tiuxetan, while $^{131}$I is labeled directly to tositumomab. The β particles emitted by radionuclides used in clinical practice, e.g. $^{90}$Y and $^{131}$I, are able to kill lymphoma cells. Differences between $^{131}$I and $^{90}$Y are listed in table I.[14] More surrounding tumors can be efficiently irradiated ('crossfire effect'), since β particles can penetrate tissue for several millimeters. The cytotoxic effects are therefore not limited to the targeted cells (figure 1).

**Table I.** Characteristics of 90-yttrium and 131-iodine

| Radionuclide | Yttrium-90 | Iodine-131 |
|---|---|---|
| Half-life | 64h | 8d |
| β-Radiation (keV) | 935 | 192 |
| γ-Radiation (keV) | – | 362 |
| Maximum tissue range (mm) | 11 | 2.9 |
| Advantages | High β energy, outpatient treatment | Easy labeling, inexpensive |
| Disadvantages | No imaging possible | High radiation for medical staff |

### 1.1 Treatment and Clinical Practice

Because of safety concerns, there are strict criteria for selection of patients suitable for RIT.[11,15] Patients must have a near-normal hemogram, and bone marrow infiltration with NHL should be ≤25%. Before treatment, dosimetry should be performed in order to keep radiation doses to normal vital organs below toxic levels.

Before administering the tracer dose for pretherapy imaging, patients are treated with unlabeled antiCD20 (rituximab in case of $^{90}$Y-ibritumomab tiuxetan and tositumomab in case of $^{131}$I-tositumomab) to prevent normal tissues, visceral sites (such as the spleen), and circulating lymphocytes from binding the radioactive dose (antigenic sink principle). This optimizes biodistribution of the radiolabeled MAbs and increases dose delivery to tumor sites.

Within hours after infusion of unlabeled antibodies, a tracer dose of the conjugated radionuclide is injected. Since $^{90}$Y is a pure β-emitter, a γ-emitting surrogate such as indium-111 ($^{111}$In) is used for single photon emission-computed tomography (SPECT) tracing of $^{90}$Y. Several γ camera scans at different timepoints need to be performed to evaluate biodistribution and dosimetry. The therapeutic procedure needs to be reconsidered if biodistribution is

1 The use of trade names is for product identification purposes only and does not imply endorsement.

unfavorable, e.g. no uptake in NHL or an unexpected high uptake in bone marrow or other organs.

In case of treatment with $^{90}$Y-ibritumomab tiuxetan, dosimetry studies (using $^{111}$In) have shown that a fixed dose related to the patient's bodyweight is feasible: 0.4 mCi/kg to a maximum of 32 mCi and 0.3 mCi/kg, or a maximum of 24 mCi if platelets are between 100 000/μL and 150 000/μL.[16,17] In this setting, its advantage is that individual dosimetry is not necessary. However, with $^{131}$I-tositumomab, clearance of the antibody will be affected by tumor size, splenomegaly, and bone marrow involvement, and these factors lead to a 4-fold variation in the effective half-life of $^{131}$I-tositumomab. Therefore, scans for dosimetry are used to determine the target dose of $^{131}$I-tositumomab. (75 cGy total body exposure, or 65 cGy if platelets are between 100 000/μL and 150 000/μL).[13]

The therapeutic dose follows the tracer dose 1–2 weeks later and should be preceded by a dose of unlabeled monoclonal antibody. Since $^{90}$Y is a pure β-emitter, in contrast to $^{131}$I, which is a β- and γ-emitter, treatment with $^{90}$Y-ibritumomab tiuxetan can be performed in an outpatient setting. If a standard dose of $^{131}$I-tositumomab (maximum total body exposure 75 cGy) is used, treatment can also be performed in an outpatient setting. If higher doses are used (>75 cGy) patients need to be hospitalized for safety reasons because of high doses of γ radiation. Also, thyroid function needs to be protected and monitored.

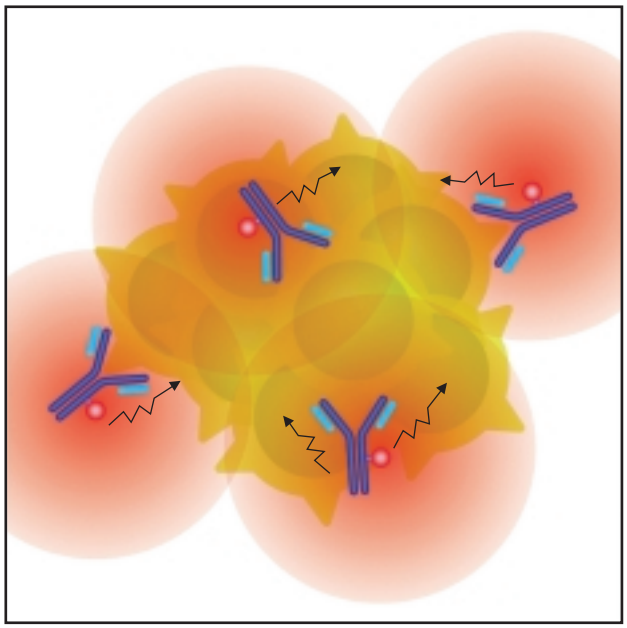

**Fig. 1.** 'Crossfire effect'. Since β particles can penetrate tissue for several millimeters, cells surrounding the cells to which the radiolabeled antibody attaches are also efficiently irradiated. Arrows indicate β-emissons penetrating unlabeled cells.

$^{90}$Y-ibritumomab tiuxetan is commercially available in Europe and the US, but $^{131}$I-tositumomab is currently only available in the US. At present, there are no data available comparing the two commercial products. Since several departments (hematology, nuclear medicine, radiology, and pharmacy) are involved in the use of RIT, multidisciplinary teamwork and coordination are prerequisites.

## 2. Feasibility and Safety Concerns

### 2.1 Toxicities and Bone Marrow Involvement

Infusion-related toxicities in RIT are mostly grade 1–2 and are associated with infusion of unlabeled monoclonal antibodies. In general, there is no organ-specific non-hematological toxicity. Bone marrow suppression is the dose-limiting RIT toxicity. Neutropenia and thrombocytopenia occur in most patients typically 7–9 weeks after treatment, which usually lasts for approximately 1–4 weeks. Physicians should be aware of the delayed course of this nadir compared with conventional chemotherapy.[11,12]

To avoid radiation damage to bone marrow progenitor cells caused by the crossfire phenomenon, in most studies patients are excluded if ≥25% of bone marrow is infiltrated by NHL. In patients treated with $^{131}$I-tositumomab with ≥25% bone marrow involvement, a clear relationship between the percentage of involvement and hematologic toxicity is seen. Patients without bone marrow disease experience less toxicity than patients with 25% bone marrow involvement, indicating that hematologic toxicity increases with the degree of bone marrow infiltration of NHL.[18]

If ≥25% of bone marrow is infiltrated and platelets are <150 000/μL, an adjusted dose of $^{131}$I tositumomab should be administered. In 11 patients with a median bone marrow involvement of 40%, a lower dose (45–55 cGy) was administered, requiring hematological support for 3 of 11 (27%) patients.[19] The authors concluded that patients with ≥25% bone marrow involvement are able to tolerate a lower total body dose of $^{131}$I-tositumomab.

### 2.2 Treatment-Related Myelodysplastic Syndromes and Acute Myeloid Leukemia

A recently published retrospective study evaluated the incidence of $^{131}$I-tositumomab treatment-related myelodysplastic syndromes (tMDS) and treatment-related acute myeloid leukemia (tAML) in 1071 patients enrolled in seven studies.[20] Of these, 995 pretreated patients had relapsed/refractory indolent NHL, with or without transformation, and 76 patients were previously untreated. After a single dose of $^{131}$I-tositumomab, baseline and post-therapy peripheral blood and marrow specimens were reviewed. tMDS/

**Table II.** Summary of clinical studies concerning treatment of patients with relapsed indolent or transformed non-Hodgkin lymphoma (NHL) with yttrium-90 ($^{90}$Y)-ibritumomab tiuxetan and iodine-131 ($^{131}$I)-tositiumomab

| Study | | Patients | Response | Reference |
|---|---|---|---|---|
| NHL | treatment | (n) | | |
| Indolent, refractory to anti-CD20 | $^{90}$Y-ibritumomab tiuxetan | 57 | 15% CR, 59% PR, duration of response 6.8mo | 25 |
| Refractory, indolent or relapsed + thrombocytopenia (100 000–150 000μL) | $^{90}$Y-ibritumomab tiuxetan | 30 | 47% CR, 36% PR, duration of response 11.5mo, median follow-up 36.5mo | 17 |
| Relapsed, indolent or transformed NHL | $^{90}$Y-ibritumomab tiuxetan vs rituximab; randomized | 143 | 80% vs 56% overall response, 30% vs 16% CR (significant), duration of response 14.2 vs 12.1mo | 26 |
| Relapsed and refractory indolent (single center) | $^{131}$I-tositiumomab | 59 | 34% CR, 37% PR | 27 |
| First/second recurrence, indolent | $^{131}$I-tositiumomab | 41 | 49% CR[u], 27% PR, overall medium duration of remission 1.3y (not reached for CR[u]) | 28 |
| Indolent, follicular large cell, transformed, progressive after rituximab | $^{131}$I-tositiumomab | 40 | 32% CR, 36% PR (panel assessed) | 29 |

**CR** = complete remission; **CR[u]** = complete remission unconfirmed; **PR** = partial remission.

tAML was reported in 35 of 995 (3.5%) previously treated patients (median follow-up 6 years from diagnosis, 2 years from RIT, annualized incidence 1.6%). Diagnoses were confirmed in 52% of the cases in a blinded review (annualized incidence 1.1%). Since chemotherapy can also cause tMDS/tAML, this incidence was expected on the basis of patients' prior treatment for NHL. No case of tMDS/tAML has been reported in the previously untreated group (follow-up 4.6 years).

In another study, after 9 years of follow-up, tMDS/tAML was demonstrated in 10 of 770 (1.3%) patients treated with $^{90}$Y-ibritumomab tiuxetan.[21] Most patients showed multiple cytogenetic aberrations, including chromosomes 5 and 7, similar to those observed after treatment with $^{131}$I-tositumomab. Long-term data are needed for final evaluation of tMDS/tAML after RIT.

## 3. Efficacy of RIT in Indolent Non-Hodgkin Lymphoma (NHL)

### 3.1 Efficacy of RIT in Relapsed, Refractory and Transformed Indolent NHL

Several clinical studies in patients with relapsed or refractory indolent NHL with $^{131}$I-tositumomab and $^{90}$Y-ibritumomab tiuxetan have been performed.[16,17,22-29] Recently, five clinical trials including 250 patients treated with tositumomab and $^{131}$I-tositumomab in patients with relapsed or refractory indolent NHL were analyzed.[7] Forty-three percent of the patients had been treated with more than four prior therapies, and 36% had not responded to their most recent therapy. Total response rates ranged from 47% to 68%, with complete response (CR) ranging from 20% to 38%. After a median follow-up of 5.3 years, 5-year progression-free survival (PFS) was 17%. Time to progression of >1 year was seen in 81 of 250 patients (32%). In this group with durable response (DR), the median duration of response was 45.8 months, and the median duration of CR had not been reached. This group also included patients with poor characteristics (bone marrow involvement, bulky disease, or transformed histology). Table II shows a summary of three clinical studies with patients treated with $^{131}$I-tositimomab.[27-29]

Re-treatment with $^{131}$I-tositumomab has been studied in 32 relapsed patients with indolent or transformed NHL who showed an initial response to $^{131}$I-tositumomab of at least 3 months.[24] RIT dose adjustments were made in the case of persistent thrombocytopenia or prior grade 4 hematologic toxicity. Overall response was 56% (median duration 15.2 months) and 25% of the patients showed CR. This was not significantly different for the first and second course of RIT. Ten of eighteen re-responders had longer responses after re-treatment.

Table II also shows a summary of clinical studies with patients treated with $^{90}$Y-ibritumomab tiuxetan.[17,25,26] Response rates ranged from 74% to 83%, CR from 15% to 47%. After a median follow-up of >3 years in a group of patients with mild thrombocytopenia, the duration of response was 11.5 months. Median overall survival has not yet been reached. Long-term responses (time to progression ≥1 year) have been seen in 14 of 30 patients (47%).[17]

Several clinical studies have been performed in patients with progressive disease who had previously received RIT. A review of studies with patients treated with $^{90}$Y-ibritumomab tiuxetan

showed that clinical responses have been achieved with all types of subsequent treatment with no apparent impact on their efficacy.[30] Also, no significant differences in toxicities with subsequent therapies have been observed between patients who had previously received RIT and those who had not. Stem cell collection for autologous stem cell transplantation after treatment with $^{90}$Y-ibriumomab tiuxetan is feasible as shown in eight patients.[31]

In conclusion, RIT induces high response rates in (pretreated) patients with indolent or transformed NHL, re-treatment is feasible (based only on $^{131}$I-tositumomab data), and a significant subgroup of patients achieve long-term DR. Data indicate that patients previously treated with RIT can undergo subsequent treatment, including stem cell transplantation, for progressive disease.

### 3.2 Efficacy of Upfront RIT in Indolent NHL

The possibility of using RIT as first-line therapy in patients with indolent NHL is of great interest. A single course of $^{131}$I-tositumomab as initial treatment for indolent lymphoma was studied in 76 patients with stage III/IV disease.[32] Patients with stable or progressive disease were eligible if bone marrow involvement was ≤25%, with an absolute neutrophil count of >1500/μL and with a platelet count of >100 000/μL. Overall response was 95%, of which 75% was CR. A molecular response was shown in 80% of assessable patients who had clinical CR. After a median follow-up of 5.1 years, the 5-year PFS for all patients was 59%, with a median PFS of 6.1 years. Of 57 patients who had a CR, 40 remained in remission for 4.3–7.7 years.

First-line combined modality treatment with RIT in combination with chemotherapy has also been investigated. Even patients with extensive bone marrow involvement at diagnosis might be eligible for RIT if chemotherapy with or without unlabeled antiCD20 is able to diminish marrow infiltration. In previously untreated patients (n = 35), RIT as first-line in combination with chemotherapy was shown to be feasible and effective with respect to PFS.[33] In this study, patients received three cycles of fludarabine, followed by tositumomab and $^{131}$I-tositumomab; this regimen produced impressive responses (100% overall response, 86% CR, 14% PR, median follow-up of 58 months). In five of six patients, bone marrow infiltration was decreased to <25% after chemotherapy, thereby enabling them to receive a standard dose of RIT. In addition, this study also demonstrated that the Follicular Lymphoma International Prognostic Index (FLIPI) was predictive for the effects of RIT: patients with FLIPI scores indicating low or intermediate risk at baseline had a significantly better PFS.[2] The authors concluded that fludarabine before RIT can decrease bone marrow involvement and even suppress HAMA responses. Recently, preliminary data show that fludarabine/mitoxantrone followed by $^{90}$Y-ibritumomab tiuxetan is highly effective in patients with untreated indolent NHL with an intermediate or high FLIPI score.[34]

These results need to be explored in future prospective studies, using risk stratification by FLIPI to select patients for RIT. Studies involving these therapeutic strategies are important for exploring the timing of RIT in the overall management of indolent lymphoma. The long natural course of follicular NHL, however, makes clinical studies for proving the effect upon overall survival time consuming.

## 4. Emerging Strategies of RIT in NHL

New therapeutic strategies in patients with indolent and aggressive NHL are under investigation concerning feasibility in patients with advanced disease, dose escalation of RIT, and combination with (high-dose) chemotherapy and stem cell transplantation. Recently, RIT was shown to be a feasible treatment for extensively pretreated patients (including myeloablative chemotherapy, without total body irradiation) with relapsed or refractory NHL.[35] Patients were treated with a standard dose of $^{90}$Y-ibritumomab tiuxetan. Grade 4 thrombocytopenia occurred in three of eight patients and neutropenia in one of eight patients. Re-treatment with RIT seems feasible after intensive pretreatment, although only minor responses were observed.

Tumor masses can be bulky in indolent lymphoma; therefore, treatment with repeated courses of RIT would be of interest in such patients. Since the crossfire phenomenon has a limited range, repeated treatment with (dose-reduced) RIT could be used for diminishing these masses over a longer period of time. Whether these new strategies are feasible and may result in improvement of overall survival in (pretreated) patients with indolent NHL needs to be determined.

To date, standard doses of RIT are used with manageable toxicity in the outpatient setting. In external beam radiotherapy for NHL, a dose-efficacy relationship is well established. To improve RIT efficacy, treatment with higher-dose RIT could be useful. In such a setting, a pretherapy imaging study and subsequent dosimetry would be particularly useful for prevention of unacceptable dose exposition to normal organs, and for evaluation of dose-response relationships. Besides SPECT cameras, positron emission tomography (PET) and PET-CT cameras are also currently widely available and can be used for this purpose. PET provides better resolution and higher sensitivity than SPECT and is also better suited for tracer quantification. To enable PET with monoclonal antibodies (immuno-PET), a suitable positron-emitting radionuclide is required. Recent studies showed that zirconium-89 ($^{89}$Zr) might be an attractive PET surrogate for $^{90}$Y. The

potential of $^{89}$Zr-immuno-PET has been demonstrated in animal models and patients, and will be further explored in clinical trials.[36]

Toxicity of high-dose RIT can be overcome by intense (clinical) hematologic supportive care, and even autologous stem cell transplantation may be considered. In aggressive NHL, high-dose RIT in combination with high-dose chemotherapy and autologous stem cell transplantation has been reported, with encouraging results.[37-40] Recently, a phase I/II study was presented, showing the feasibility of high-dose $^{90}$Y-ibritumomab tiuxetan (up to doses 4-fold higher than standard, i.e. 1.5 mCi/kg) with peripheral blood stem cell support. This treatment could be safely delivered in elderly and heavily pretreated patients, including those who previously received high-dose chemotherapy.[41] High-dose RIT in combination with chemotherapy and autologous stem cell transplantation in patients with aggressive NHL is feasible and does not show increased toxicity compared with standard conditioning regimens.[42] With this strategy, the dose of radiation delivered to the tumor can be 10-fold higher than the dose achievable with total body irradiation. Whether this increase in radiation dose will produce more durable remissions and an improvement in overall survival in patients with aggressive NHL needs to be determined.

## 5. Conclusion

RIT is part of standard care for patients with relapsed or refractory indolent NHL, although the exact status and timing of the treatment needs to be determined. RIT as first-line treatment, with or without chemotherapy, seems very promising. Clinical efficacy has been proved in several studies with relapsed or refractory NHL with respect to response and increase of PFS. In a subgroup of patients, long-term durable responses have been seen. RIT is feasible in heavily pretreated patients and does not compromise future treatments in case of progressive disease. Randomized, prospective phase III studies are in progress to show whether upfront RIT, rather than RIT in second- or third-line treatment or after failure of therapy with unlabeled monoclonal antibodies, would be more efficient. The encouraging results of RIT in indolent NHL initiated new investigations focusing on its benefit for patients with aggressive NHL.

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Correspondence and offprints: Dr *Otto J. Visser*, Department of Hematology, VU University Medical Center, BR 240, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.
E-mail: o.visser@vumc.nl

# Preparation and evaluation of $^{89}$Zr-Zevalin for monitoring of $^{90}$Y-Zevalin biodistribution with positron emission tomography

Lars R. Perk[1], Otto J. Visser[2], M. Stigter-van Walsum[1], Maria J. W. D. Vosjan[1], Gerard W. M. Visser[3], Josée M. Zijlstra[2, 3], Peter C. Huijgens[2], Guus A. M. S. van Dongen[1, 3]

[1] Department of Otolaryngology/Head and Neck Surgery, VU University Medical Centre, De Boelelaan 1117, P.O. Box 7057, 1007 MB, Amsterdam, The Netherlands
[2] Department of Hematology, VU University Medical Centre, Amsterdam, The Netherlands
[3] Department of Nuclear Medicine and PET Research, VU University Medical Centre, Amsterdam, The Netherlands

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**Abstract.** *Purpose:* To evaluate whether $^{89}$Zr can be used as a PET surrogate label for quantification of $^{90}$Y-ibritumomab tiuxetan ($^{90}$Y-Zevalin) biodistribution and dosimetry before myeloablative radioimmunotherapy.
*Methods:* Zevalin was labelled with $^{89}$Zr by introducing *N*-succinyldesferal (*N*-sucDf) as a second chelate. For comparison of the in vitro stability of $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin (as a substitute for $^{90}$Y), samples were incubated in human serum at 37°C up to 6 days. Biodistribution of $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin was assessed at 24, 48, 72 and 144 h p.i. by co-injection in nude mice bearing the non-Hodgkin's lymphoma (NHL) xenograft line Ramos. The clinical performance of $^{89}$Zr-Zevalin-PET was evaluated via a pilot imaging study in a patient with NHL, who had undergone [$^{18}$F]FDG-PET 2 weeks previously.
*Results:* Modification of Zevalin with *N*-sucDf resulted in an *N*-sucDf-to-antibody molar ratio of 0.83±0.04. After radiolabelling and purification, the radiochemical purity and immunoreactivity of $^{89}$Zr-Zevalin always exceeded 95% and 80%, respectively. $^{89}$Zr-Zevalin showed the same stability in serum as $^{88}$Y-Zevalin, with a radiochemical purity >95% during a period of 6 days. The co-injected $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin conjugates showed a very similar biodistribution, except for liver and bone accumulation at 72 and 144 h p.i., which was significantly higher for $^{89}$Zr than for $^{88}$Y. PET images obtained after injection of $^{89}$Zr-Zevalin showed clear targeting of all known tumour lesions.
*Conclusion:* $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin showed a very similar biodistribution in mice, implying that $^{89}$Zr-Zevalin-PET might be well suited for prediction of $^{90}$Y-Zevalin biodistribution in a myeloablative setting.

*Keywords:* Immuno-PET – Radioimmunotherapy – Ibritumomab tiuxetan – Zirconium-89 – Yttrium-90

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Guus A. M. S. van Dongen (✉)
Department of Otolaryngology/Head and Neck Surgery,
VU University Medical Centre,
De Boelelaan 1117,
P.O. Box 7057, 1007 MB, Amsterdam, The Netherlands
e-mail: gams.vandongen@vumc.nl
Tel.: +31-20-4440953, Fax: +31-20-4443688

## Introduction

The yttrium-90 ($^{90}$Y) labelled anti-CD20 murine monoclonal antibody (MAb) ibritumomab tiuxetan ($^{90}$Y-Zevalin, Biogen IDEC and Schering AG) was approved by the U.S. Food and Drug Administration in 2002 for the treatment of patients with relapsed or refractory low-grade, follicular or transformed B-cell non-Hodgkin's lymphoma (NHL), including rituximab-refractory follicular NHL [1, 2]. In 2004, the European Medicines Agency (EMEA) approved $^{90}$Y-Zevalin for the treatment of adult patients with rituximab-relapsed or refractory CD20+ follicular B-cell NHL [3]. The Zevalin radioimmunotherapy (RIT) procedure is preceded by administration of 250 mg/m$^2$ rituximab (Rituxan, Biogen IDEC and Genentech) to clear peripheral B cells and to improve biodistribution of the radiolabelled Zevalin. Recently, promising results have also been obtained in the treatment of aggressive NHL. In these studies, high-dose $^{90}$Y-Zevalin RIT was added to high-dose chemotherapy followed by autologous stem cell transplantation (AuSCT) [4, 5]. The optimal use of $^{90}$Y-Zevalin in this kind of myeloablative RIT, however, remains to be determined.

$^{90}$Y has a physical half-life of 64.1 h and emits high-energy $\beta^-$ particles. The absence of $\gamma$-ray emission minimises dose radiation burden for medical personnel and

relatives and enables outpatient treatment. Whereas these characteristics make $^{90}$Y attractive for therapy, the lack of associated photon emission does not allow external imaging of the in vivo distribution of the $^{90}$Y-labelled MAb. Therefore, to confirm tumour targeting and to estimate the absorbed dose (dosimetry) by tumours as well as normal tissues, in the studies on myeloablative RIT a pretherapy single-photon emission computed tomography (SPECT) imaging study with indium-111 ($^{111}$In, $t_{1/2}$=67.3 h) labelled Zevalin was performed [4, 5]. For coupling of $^{111}$In and $^{90}$Y to Zevalin, the chelator 1-isothiocyanatobenzyl-3-methyldiethylene triamine penta-acetic acid designated MX-DTPA (tiuxetan) was used, because it binds these three-valent radionuclides with high stability.

In myeloablative RIT, high doses of $^{90}$Y are used and therefore dosimetry as accurate as possible will be highly desirable for prevention of exposure of normal organs to unacceptable doses and for evaluation of dose-response relationships. Positron emission tomography (PET) is better suited than SPECT for tracer quantification [6], while targeting information can be combined with anatomical information when PET-CT is used.

Visualisation and quantification of MAb biodistribution with a PET camera (immuno-PET) requires a suitable positron-emitting radionuclide. As a potential PET surrogate for $^{90}$Y, the positron emitter $^{86}$Y is receiving attention [7, 8]. An advantage in the use of the same element would be that one type of chelator can be used for coupling of both isotopes. $^{86}$Y, however, emits prompt gammas, which can hamper accurate quantification; furthermore, its half-life of 14.7 h is relatively short for optimal imaging and dosimetry with an intact MAb like Zevalin, as typically 2–4 days are required to achieve optimal tumour to non-tumour ratios. The positron emitter zirconium-89 ($^{89}$Zr) might be a better candidate for prediction and monitoring of the biodistribution of $^{90}$Y in RIT studies, especially because of its long half-life of 78.4 h. Procedures for the production and purification of large batches of $^{89}$Zr have recently been established at our institute. A versatile method for stable coupling of $^{89}$Zr to MAbs, including MAb U36, which is at an early stage of development for detection of head and neck cancer, was developed using the desferal-chelate precursor tetrafluorophenol-*N*-succinyl-desferal-Fe (TPF-*N*-sucDf-Fe) [9]. Preliminary clinical evaluation of $^{89}$Zr-MAb U36 revealed that tumour deposits can be clearly visualised after administration of 74 MBq $^{89}$Zr [6]. Moreover, in nude mice bearing head and neck cancer xenografts, $^{89}$Zr-*N*-sucDf-U36 and $^{88}$Y-DOTA-U36 ($^{88}$Y as a substitute for $^{90}$Y) conjugates showed a highly similar biodistribution, while accurate $^{89}$Zr quantification appeared feasible, these being prerequisites to justify the use of $^{89}$Zr-immuno-PET for scouting $^{90}$Y-RIT [10].

Therefore, introduction of $^{89}$Zr-Zevalin-PET in high-dose $^{90}$Y-Zevalin RIT might be an attractive option for prediction of $^{90}$Y-Zevalin biodistribution and dosimetry, and of toxicity and tumour response upon high-dose $^{90}$Y-Zevalin RIT in a myeloablative setting. To enable the use of $^{89}$Zr-Zevalin in such a setting, the present study was conducted to develop a route to an $^{89}$Zr-Zevalin conjugate capable of monitoring the biodistribution of the therapeutic $^{90}$Y-Zevalin conjugate in the clinic. Since only MX-DTPA premodified ibritumomab is available for clinical use, and MX-DTPA does not bind the four-valent $^{89}$Zr, *N*-sucDf was coupled as a second chelate to Zevalin. The resulting newly developed double-chelator modified $^{89}$Zr-Zevalin conjugate was measured for stability in serum and for preservation of immunoreactivity, including during storage. For the analysis of the pharmacokinetic behaviour, the biodistribution of $^{89}$Zr-Zevalin was compared with that of $^{88}$Y-Zevalin upon co-injection in NHL xenograft-bearing nude mice. The preliminary clinical performance of $^{89}$Zr-Zevalin-PET was evaluated in a patient with CD20+ B-cell NHL.

## Materials and methods

### *Monoclonal antibody, radioactivity and cell lines*

The monoclonal antibody ibritumomab tiuxetan (Zevalin) was obtained from Schering Nederland BV, The Netherlands. $^{89}$Zr (2.7 GBq/ml in 1 *M* oxalic acid) was produced by the BV Cyclotron by a (p,n) reaction on natural $^{89}$Y and isolated with the use of a hydroxamate column [9]. $^{88}$Y (37 MBq/ml in 0.1 *M* HCl) was obtained from Isotope Products Europe, and $^{90}$Y (18.5 GBq/ml in 0.05 *M* HCl) was obtained from PerkinElmer. The CD20+ B-cell lymphoma cell line Ramos was obtained from the American Type Culture Collection (ATCC number CRL-1596).

### *Radiolabelling*

Labelling of Zevalin with $^{89}$Zr was achieved starting from the chelate TFP-*N*-sucDf-Fe, as described previously [9]. In short, the TFP-*N*-sucDf-Fe ester was coupled to the lysine residues of Zevalin (1.6 mg). After removal of Fe(III) by transchelation to ethylene diamine tetra-acetic acid (EDTA) and purification on a PD10 column (Amersham Biosciences; eluent: 0.9% NaCl/gentisic acid 5 mg/ml, pH 5.0), the premodified Zevalin was labelled with $^{89}$Zr in 0.5 *M N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES) buffer at pH 7.0 to arrive at a specific activity of approximately 74 MBq/mg. Finally, $^{89}$Zr-Zevalin was purified on a PD10 column (eluent: 0.9% NaCl/gentisic acid 5 mg/ml, pH 5.0) to remove unbound $^{89}$Zr. A schematic representation of $^{89}$Zr-Zevalin (i.e. $^{89}$Zr-*N*-sucDf-ibritumomab tiuxetan) in comparison with the routinely used $^{90}$Y-Zevalin conjugate is shown in Fig. 1.

$^{90}$Y-Zevalin was prepared according to the instructions of the supplier entitled: "Preparation and dispensing information on $^{90}$Y and $^{111}$In-ibritumomab tiuxetan". With this procedure, $^{90}$Y was coupled to Zevalin at a specific activity of approximately 740 MBq/mg MAb, and formulated in a phosphate-buffered saline (PBS) containing 7.5% human serum albumin (HSA) and 1 m*M* diethylene triamine penta-acetic acid (DTPA), pH 7.2 (formulation buffer). Zevalin was labelled with $^{88}$Y according to the same procedure, but with reaction volume modifications.

### *Analyses*

Conjugates were analysed by instant thin-layer chromatography (ITLC) and high-performance liquid chromatography (HPLC) for radiochemical purity, by sodium dodecylsulphate-polyacrylamide gel

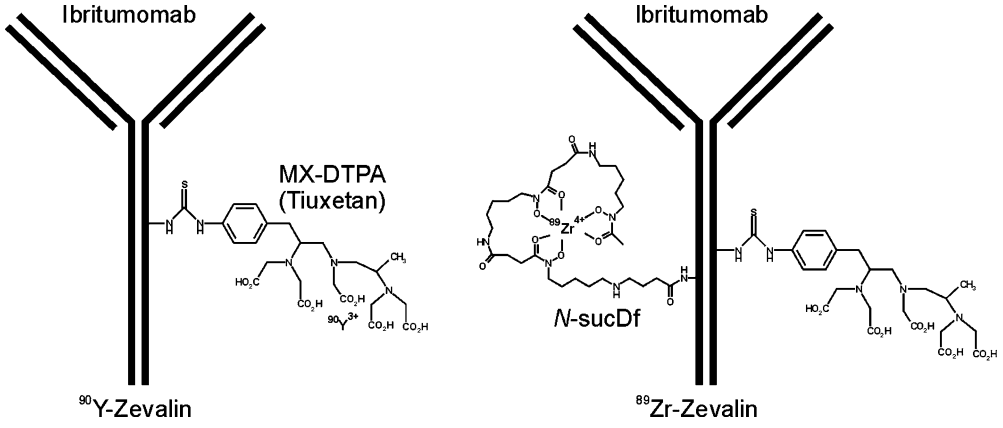


**Fig. 1.** Schematic representation of $^{90}$Y-labelled Zevalin, consisting of the MAb ibritumomab and the chelate MX-DTPA (*left*), and of $^{89}$Zr-labelled Zevalin, consisting of the MAb ibritumomab and the chelates MX-DTPA (empty) as well as the chelate *N*-sucDf used for complexation of $^{89}Zr^{4+}$ (*right*)

electrophoresis (SDS-PAGE) followed by phosphor imager analyses for integrity, and by a cell-binding assay for immunoreactivity. ITLC analysis of radiolabelled Zevalin was performed on silica gel impregnated glass fibre sheets (Gelman Sciences). As the mobile phase, 20 m*M* citrate buffer pH 5.0 was used for $^{89}$Zr-Zevalin and 0.9% NaCl for $^{88}$Y- and $^{90}$Y-Zevalin. Antibody-bound $^{89}$Zr, $^{88}$Y and $^{90}$Y remained at the origin ($R_f$ 0.0), whereas unreacted $^{89}$Zr, $^{88}$Y and $^{90}$Y (as $^{89}$Zr-citrate or $^{88}$Y/$^{90}$Y-DTPA) moved with $R_f$ 0.8–1.0. HPLC monitoring of the synthesis of $^{89}$Zr-Zevalin and of the labelling to arrive at $^{88}$Y/$^{90}$Y-Zevalin was performed on a Jasco HPLC system using a Superdex 200 10/300 GL size exclusion column (Amersham Biosciences). As eluent, a mixture of 0.05 *M* sodium phosphate/0.15 *M* NaCl (pH 6.8) was used at a flow rate of 0.5 ml/min.

The *N*-sucDf-to-MAb molar ratio was quantified using HPLC analysis with TFP-*N*-sucDf-Fe ester spiked with $^{59}$Fe in the modification step, and measuring the amount of MAb bound *N*-sucDf-$^{59}$Fe and unreacted *N*-sucDf-$^{59}$Fe.

Single isotope counting was performed with a γ-well counter (Compugamma, LKB Wallac) for $^{89}$Zr, $^{88}$Y and $^{59}$Fe, and with a β-counter (Rackbeta, LKB Wallac) for $^{90}$Y using Čerenkov radiation. The 511-keV γ-energy of $^{89}$Zr and the 1,837-keV γ-energy of $^{88}$Y were used for dual-isotope counting in the biodistribution study. Crossover corrections from one radionuclide into the alternate window were performed using a standard of each radionuclide. When $^{89}$Zr was measured with the γ-well counter in the presence of $^{90}$Y, a similar procedure was applied to correct for background caused by $^{90}$Y bremsstrahlung. When $^{90}$Y was measured with the β-counter in the presence of $^{89}$Zr, corrections were applied to adjust for background caused by $^{89}$Zr.

Gel electrophoresis was performed on a Pharmacia Phastgel System (Amersham Biosciences) using preformed 7.5% SDS-PAGE gels (Amersham Biosciences) under non-reducing conditions. In vitro binding characteristics of radiolabelled Zevalin were determined in an immunoreactivity assay essentially as described by Lindmo et al. [11], using Ramos cells fixed with 2.0% paraformaldehyde. Data were graphically analysed in a modified Lineweaver-Burk (double-reciprocal) plot and the immunoreactivity was determined by extrapolating to conditions representing infinite antigen excess.

### *In vitro stability of radiolabelled Zevalin*

Three sets of stability tests were performed, each with a particular design, of relevance for the clinical studies planned: For validation of $^{89}$Zr labelling in compliance with Good Manufacturing Practice (GMP), three clinical batches of $^{89}$Zr-Zevalin were produced and quality tests were performed. To this end, $^{89}$Zr-Zevalin (1.8 mg, 74 MBq) was diluted in 20 ml 0.9% NaCl/gentisic acid 5 mg/ml, pH 5.0 (clinical formulation) and incubated at 4°C or room temperature for 48 h. At 0, 24 and 48 h, aliquots were taken and analysed by ITLC as well as HPLC. In addition, the immunoreactivity was assessed at these time points.

In forthcoming clinical myeloablative RIT studies, we anticipate the possibility of administration of $^{89}$Zr-Zevalin alone for pretherapy imaging as well as administration in combination with high-dose $^{90}$Y-Zevalin (for RIT). In the latter case, the PET and RIT conjugate might be administered together as a mixture. For testing the stability of both conjugates in such a mixture at challenging radioactivity concentrations, 37 MBq $^{89}$Zr-Zevalin (0.5 mg) and 720 MBq $^{90}$Y-Zevalin (1 mg) in formulation buffer (PBS buffer containing 7.5% HSA and 1 m*M* DTPA, pH 7.2) were combined in a final volume of 6.5 ml and stored at 4°C. At various time intervals (2, 4, 7 and 24 h), samples were taken for quality testing of both conjugates, including ITLC, HPLC and binding assay.

For testing the in vitro stability in human serum of $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin, samples of 0.1 ml containing the radiolabelled MAbs were added to 0.9 ml of freshly prepared human serum and incubated at 37°C in a humidified incubator maintained at 5% $CO_2$ and 95% air. In this case, $^{88}$Y was used instead of $^{90}$Y to facilitate quantification in serum. At various time intervals (1, 2, 4, 6 days), aliquots were taken and analysed by ITLC and HPLC.

### *Biodistribution*

Nude mice bearing the subcutaneously implanted B-cell lymphoma cell line Ramos were used. Female mice (athymic *nu/nu*, 21–31 g, Harlan CPB) were 10–14 weeks old at the time of the experiment. All animal experiments were performed according to National Institutes of Health principles of laboratory animal care and Dutch national law ("Wet op de dierproeven", Stb 1985, 336).

The mice ($n$=16) were injected intravenously with a mixture of 0.37 MBq $^{89}$Zr-Zevalin and 0.13 MBq $^{88}$Y-Zevalin. Unlabelled Zevalin was added to this mixture so that all animals received 100 μg MAb in total. At 24, 48, 72 and 144 h post injection, groups of four mice were anaesthetised, bled, killed and dissected. After blood, tumour, normal tissues and gastrointestinal contents had been weighed, the amount of radioactivity in each was measured in a γ-well counter. Radioactivity uptake was calculated as the percentage of the injected dose per gram of tissue (%ID/g). Differences in tissue uptake between co-injected conjugates were statistically analysed for each time point with SPSS 11.0 software using Student's $t$ test for paired data. Two-sided significance levels were calculated and $p<0.05$ was considered statistically significant.

*PET imaging*

The preliminary clinical performance of $^{89}$Zr-Zevalin PET was evaluated in a male patient (aged 49 years) who had an indolent CD20+ NHL, with transformation to diffuse large B-cell NHL, with relapsed disease after AuSCT. This feasibility study, ahead of the planned study "$^{90}$Y-Zevalin myeloablative RIT and $^{89}$Zr-Zevalin-PET in conditioning for stem cell transplantation in patients with aggressive B-cell NHL", was reviewed and approved by the Medical Ethics Committee of the VU University Medical Centre. The patient gave written informed consent after receiving a thorough explanation of the study.

PET scanning was performed using a dedicated full ring PET scanner (ECAT EXACT HR+, CTI/Siemens). A PET session with $^{89}$Zr-Zevalin was performed to assess biodistribution and to confirm targeting of tumour lesions that had been delineated 2 weeks previously by [$^{18}$F]fluorodeoxyglucose (FDG) PET scanning.

In the case of $^{89}$Zr-Zevalin immuno-PET, whole-body scans were performed, consisting of seven bed positions covering the patient from the base of the skull to the upper femur. At each bed position a 3-min transmission scan, acquired using three germanium-68 rod sources, and a 7-min emission scan in 3D mode were acquired. The patient first received rituximab, 250 mg/m$^2$ over 3.5 h, followed within 4 h by 74 MBq $^{89}$Zr-Zevalin. The patient received a total of 1.8 mg Zevalin by adding unlabelled Zevalin. Whole-body scans were performed at 2 and 96 h after intravenous injection of $^{89}$Zr-Zevalin. All scans were normalised and corrected for randoms, scatter, attenuation and decay. Reconstructions were performed using an attenuation- and normalisation-weighted ordered subset expectation maximisation (OSEM) algorithm (ECAT software, version 7.2, CTI/Siemens) with two iterations and 16 subsets followed by post-smoothing of the reconstructed image using a 5-mm FWHM Gaussian filter. OSEM reconstructions without attenuation correction were performed as well. Interpretation of the scans was performed using these non-attenuation-corrected images and was based on asymmetry and retention of activity on the late image.

[$^{18}$F]FDG-PET was performed essentially as described previously [12]. In short, the patient was required to fast for at least 6 h before the scan. A whole-body scan was performed consisting of eight bed positions from the crown to the midfemur. At each bed position a 7-min emission scan in 2D mode was performed. Scanning started about 60 min after intravenous injection of 400 MBq [$^{18}$F]FDG (BV Cyclotron, The Netherlands). The scan was corrected in the same way as described above for the $^{89}$Zr-Zevalin scans; however, no attenuation correction was performed. The PET image was read visually using standard ECAT software: foci with increased uptake versus background were considered abnormal, taking physiological biodistribution of [$^{18}$F]FDG into account.

## Results

*Modification and radiolabelling*

Labelling of Zevalin-MX-DTPA with $^{89}$Zr resulted in a labelling yield below 0.1%, confirming the lack of affinity of $^{89}$Zr for DTPA chelates. Modification of Zevalin with the chelate TFP-*N*-sucDf-Fe resulted in a reproducible *N*-sucDf-to-MAb molar ratio of 0.83±0.04 (mean±SD, *n*=8). Subsequent labelling of *N*-sucDf-Zevalin with $^{89}$Zr resulted in overall labelling yields of >60% and radiochemical purity always exceeded 95% after purification on PD10. Labelling of Zevalin with $^{88}$Y or $^{90}$Y resulted in overall labelling yields of >96% and the radiochemical purity was more than 96% for both products. Immunoreactivity of $^{89}$Zr-Zevalin was more than 80% at infinite antigen excess and similar to the immunoreactivity of $^{88}$Y/$^{90}$Y-labelled Zevalin conjugates. No physical evidence of antibody degradation was observed for any of the three products, as determined by SDS-PAGE followed by phosphor imager analyses.

*In vitro stability testing*

The in vitro stability was evaluated by three sets of experiments: The results of the first set, the validation productions according to GMP procedures, are shown in Table 1. $^{89}$Zr-Zevalin formulated in 20 ml 0.9% NaCl/gentisic acid 5 mg/ml (pH 5.0) can be stored for 48 h at 4°C and also at room temperature without decrease in radiochemical purity to a level below 95%. Immunoreactivity of the conjugate remained above 80% when stored at 4°C for 48 h and at room temperature for 24 h. This procedure resulted in a sterile final product with endotoxin levels <1.28 EU/ml (acceptance level <5 EU/ml).

In the second set, $^{89}$Zr-Zevalin and $^{90}$Y-Zevalin were joined in a formulation mixture to observe the damaging effect of radiolysis upon storage at high radioactivity concentration (111 MBq $^{90}$Y/ml). The radiochemical purity and immunoreactivity of both products were determined at various time points. Upon storage at 4°C for 4 h, the radiochemical purity of $^{89}$Zr-Zevalin remained >95%. At 24 h it decreased to 94.3%. The radiochemical purity of $^{90}$Y-Zevalin remained >96% during 24 h. For both products, immunoreactivity remained above 80% during this period. These data indicate that both conjugates will meet the clinical release specification as set for $^{90}$Y-Zevalin (radiochemical purity >95%), when the mixture of $^{89}$Zr-Zevalin with high-dose $^{90}$Y-Zevalin is administered to the patient within 4 h after production and formulation.

In the third set, $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin were individually tested for stability in human serum. Both

**Table 1.** In vitro stability of clinically formulated $^{89}$Zr-Zevalin

| Incubation time (h) | Radiochemical purity (%)[a] | |
|---|---|---|
| | ITLC | HPLC |
| 0 | 97.3±0.9 | 96.1±0.6 |
| 4 (4°C) | 97.2±0.4 | 96.1±1.0 |
| 4 (RT) | 96.6±0.5 | 96.7±1.3 |
| 24 (4°C) | 97.2±0.3 | 96.5±1.6 |
| 24 (RT) | 97.0±0.1 | 97.4±1.0 |
| 48 (4°C) | 97.3±0.3 | 96.8±0.3 |
| 48 (RT) | 96.1±0.5 | 95.6±1.0 |

[a]At each time point, three samples were analysed for radiochemical purity by ITLC and HPLC

*RT* room temperature

conjugates were stable in serum (radiochemical purity > 95%) for a period of at least 6 days.

*Biodistribution*

For comparison of the biodistribution of $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin, the two conjugates were co-injected in nude mice bearing Ramos B-cell tumours. At 24, 48, 72 and 144 h post injection, the average uptake (%ID/g, mean± SD) in tumour, blood, normal tissues and gastrointestinal contents was determined. These results are shown in Fig. 2. In general, $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin showed similar uptake in tumour and most other organs at all time points. Significant differences ($p<0.05$) between the two conjugates were found at 72 and 144 h after injection in liver, thigh bone and sternum. Tumour uptake levels of $^{89}$Zr-Zevalin were 17.0±7.6%ID/g at 24 h, 20.9±4.4%ID/g at 48 h, 19.6±4.1%ID/g at 72 h and 9.8±2.5%ID/g at 144 h; for $^{88}$Y-Zevalin these levels were 16.7±8.3%ID/g at 24 h, 20.6±5.7%ID/g at 48 h, 20.7±4.6%ID/g at 72 h and 10.0± 2.5%ID/g at 144 h. Blood levels slowly decreased from

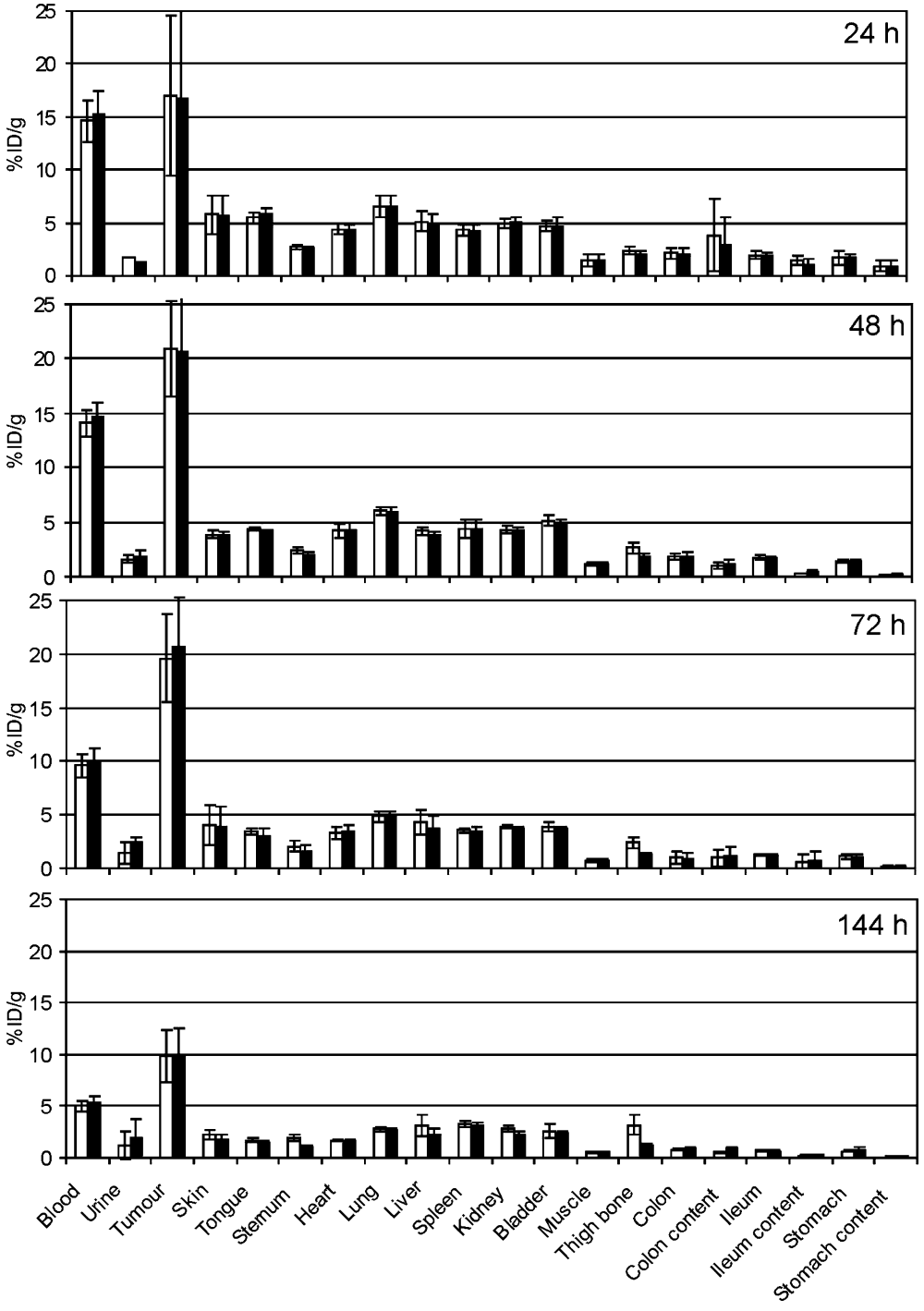


**Fig. 2.** Biodistribution of co-injected $^{89}$Zr-Zevalin (*white bars*) and $^{88}$Y-Zevalin (*black bars*) in nude mice bearing the B-cell lymphoma cell line Ramos at 24, 48, 72 and 144 h after injection

14.6±2.0%ID/g at 24 h to 5.0±0.5%ID/g at 144 h for $^{89}$Zr-Zevalin and from 15.2±2.2%ID/g at 24 h to 5.4±0.6%ID/g at 144 h for $^{88}$Y-Zevalin.

*PET imaging*

For evaluation of the clinical performance of $^{89}$Zr-Zevalin, a pilot PET imaging study (without RIT) was conducted in a patient with CD20+ B-cell NHL. The patient had undergone [$^{18}$F]FDG-PET scanning previously, the scan indicating cervical, mediastinal, left caput humeri, splenic, para-aortic and inguinal tumour involvement (Fig. 3a, tumour lesions are indicated by arrows). Whole-body images obtained 96 h after administration of $^{89}$Zr-Zevalin revealed clear uptake of the conjugate in all tumour lesions (Fig. 3b). No increased uptake in normal organs was observed except for the liver. None of the tumour sites were clearly delineated on the early $^{89}$Zr-Zevalin-PET image (2 h p.i.), which showed mainly blood pool activity (diminishing over time) with visualisation of nose, heart, lungs, liver and spleen (Fig. 4).

## Discussion

An important issue in RIT is confirmation of tumour targeting and assessment of dose delivery, especially in high-dose RIT studies. This might be accomplished by performing an immuno-PET imaging procedure prior to RIT. At least three requirements need to be met for optimal use of an imaging radioimmunoconjugate as a predictor of the biodistribution of a therapeutic radioimmunoconjugate. First, imaging and RIT conjugate should have a similar biodistribution. Second, radionuclides used for imaging and RIT should have similar physical half-lives, preferably matching with the biological half-life of the MAb (typically 2–4 days for intact MAbs). Third, procedures for quantification of uptake and subsequent dose calculations should be reasonably accurate.

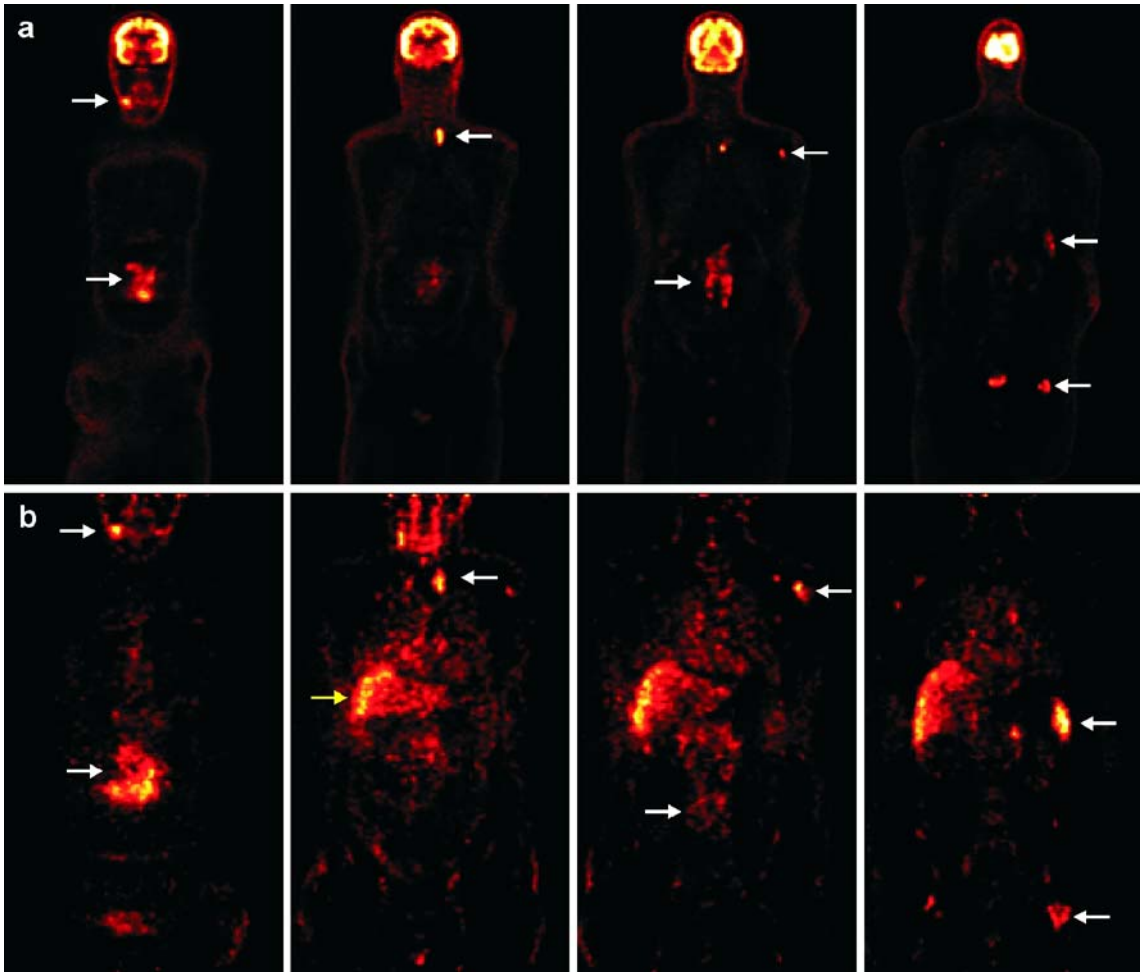


**Fig. 3. a** [$^{18}$F]FDG-PET scan of the NHL patient. Coronal images from anterior (*left*) to posterior (*right*), with visualisation of cervical, mediastinal, left caput humeri, splenic, para-aortic and inguinal lymphomas. Localisations are indicated by *white arrows*. **b** $^{89}$Zr-Zevalin immuno-PET scan 96 h p.i. of the same NHL patient. Coronal images from anterior (*left*) to posterior (*right*); slices correspond to those of the [$^{18}$F]FDG-PET scan. Tumour localisations are indicated by *white arrows*. Note targeting of tumour localisations also visualised by [$^{18}$F]FDG-PET. Liver uptake (*yellow arrow*) is probably due to retention of $^{89}$Zr after catabolism of the conjugate

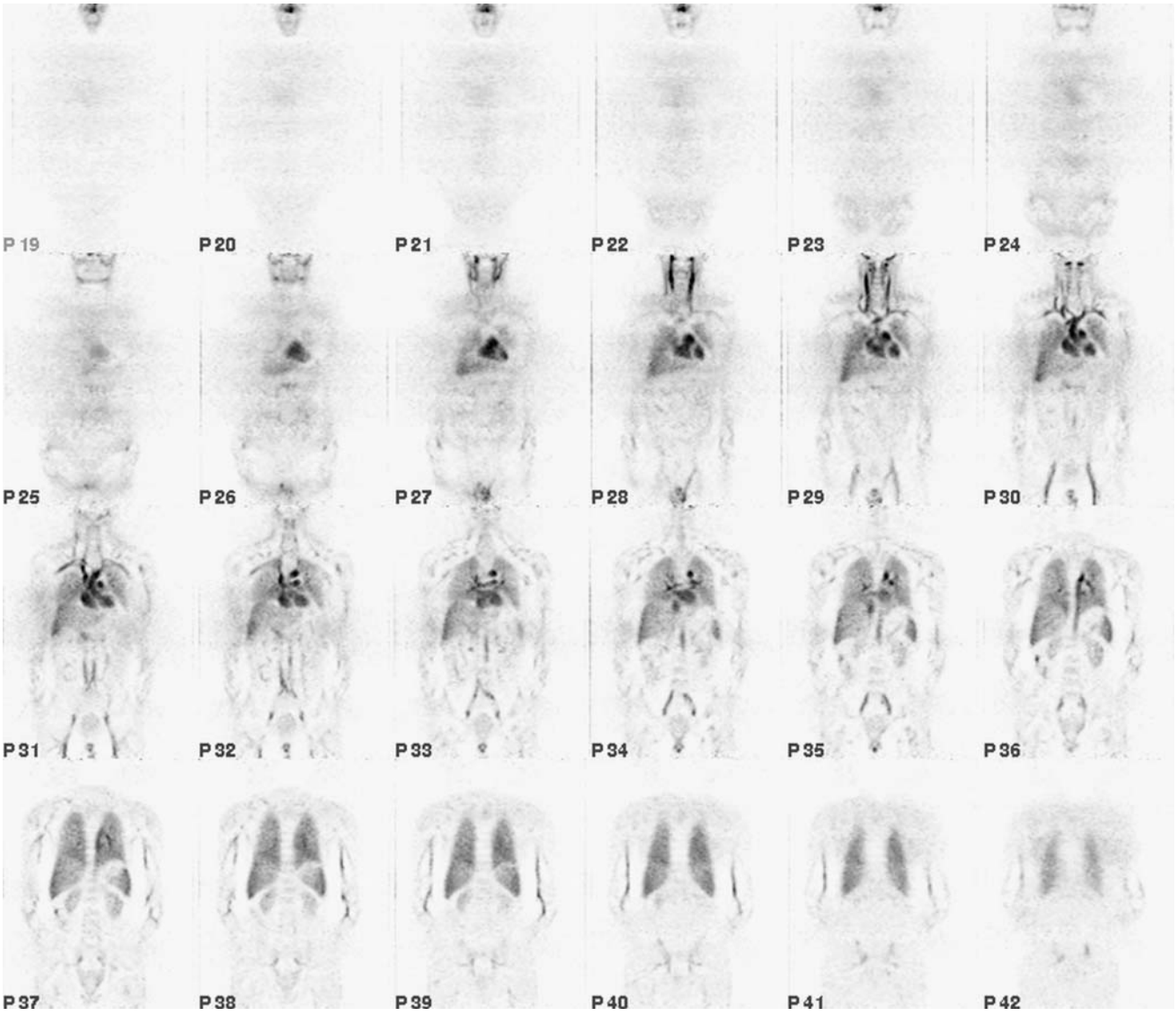


**Fig. 4.** $^{89}$Zr-Zevalin immuno-PET scan 2 h p.i. Coronal images from anterior (*upper left*) to posterior (*lower right*), showing mainly blood pool activity with visualisation of nose, heart, lungs, liver and spleen

The choice of the positron emitter is an important factor for a successful pretherapy scouting procedure with PET. Only two long-lived positron emitters seem well suited for imaging intact MAbs, namely $^{89}$Zr ($t_{1/2}$=78.4 h) and iodine-124 ($^{124}$I, $t_{1/2}$=100.3 h). Of these isotopes, $^{89}$Zr can be obtained in high yield and with high radionuclidic purity by a (p,n) reaction on $^{89}$Y, which is an attractive target material because of its 100% natural abundance [9]. As a result, production costs are relatively low. Moreover, $^{89}$Zr has no prompt gammas that can hamper image quality and accurate quantification [10]. Recent studies at our institute revealed that $^{89}$Zr residualises after catabolism, a phenomenon also observed with $^{90}$Y, $^{111}$In and lutetium-177 ($^{177}$Lu) [13]. Such residualisation is not observed with, for example, rhenium-186 ($^{186}$Re), $^{131}$I or $^{124}$I [14], and therefore $^{124}$I-Zevalin cannot be used for monitoring $^{90}$Y-Zevalin biodistribution.

$^{89}$Zr cannot be stably bound by the chelate MX-DTPA. Therefore, we coupled a second chelate, *N*-sucDf, to Zevalin. The *N*-sucDf-to-MAb molar ratio was chosen to be kept below 1, since one cannot unlimitedly modify lysine groups without alteration of the biodistribution [15, 16]. Subsequent labelling of this double-chelator modified conjugate with $^{89}$Zr resulted in reproducible labelling yields and specific activities of at least 74 MBq/mg Zevalin, while preserving immunoreactivity and integrity of the MAb. Validation productions of $^{89}$Zr-Zevalin according to GMP showed reproducibility of labelling procedures, and the possibility of storing $^{89}$Zr-Zevalin in a clinical formulation for 48 h at 4°C and for 24 h at room temperature without unacceptable loss of radiochemical purity and immunoreactivity. In addition, we joined $^{89}$Zr-Zevalin and $^{90}$Y-Zevalin in a formulation mix at clinical dose concentration. The high β-energy of $^{90}$Y can result in

radiolytic damage to the radiolabelled MAbs. $^{89}$Zr-Zevalin remained stable in this formulation solution, without impairment of immunoreactivity. This offers the flexibility to administer both conjugates either together in one single mixture, or separately directly after each other. Preceding the biodistribution study, the in vitro stability of $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin in human serum was analysed. Stability of both conjugates under these conditions was comparable and high, and radiochemical purity was always above 95% for both radioimmunoconjugates over a period of 6 days. These results indicate that $^{89}$Zr had been coupled in a stable way to Zevalin, without affecting the quality of the MAb.

To demonstrate that the newly developed double-chelator modified Zevalin labelled with $^{89}$Zr can be used for localisation of the therapeutic Zevalin labelled with $^{90}$Y, a biodistribution study was conducted in nude mice bearing Ramos B-cell tumours. This study revealed that $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin have a very comparable distribution (Fig. 2). It is of note that the apparent loss (in %ID/g) of the residualising $^{89}$Zr and $^{88}$Y in tumours at 144 h post injection relative to earlier time points is most probably the result of fast tumour growth, since the mean tumour masses at the time of dissection were 192±68 mg at 72 h compared with 406±162 mg at 144 h. Nevertheless, there were some differences ($p$<0.05) between $^{89}$Zr-Zevalin and $^{88}$Y-Zevalin in liver and bone at later time points. This difference in uptake in these organs was in the same range as previously observed in biodistribution studies with $^{89}$Zr- and $^{88}$Y-labelled MAbs [10, 13]. Differences in biodistribution were also reported between $^{111}$In- and $^{90}$Y-labelled Zevalin [17], with, for example, a difference of 1.7±0.3 versus 3.2±0.3% ID/g in bone at 72 h post injection for $^{111}$In-Zevalin versus $^{90}$Y-Zevalin, respectively. Deviating bone uptake is probably due to subtle differences in the in vivo stability of the $^{111}$In- and $^{90}$Y-DTPA complexes [18].

In the case of myeloablative RIT, where bone marrow toxicity is not dose limiting, $^{89}$Zr-Zevalin seems well suited to predict $^{90}$Y-Zevalin dosimetry. For prediction of dose delivery to bone marrow in non-myeloablative RIT, $^{89}$Zr-immuno-PET seems unsuitable owing to differing bone uptake of $^{89}$Zr and $^{90}$Y. Marrow dosimetry in this setting is challenging anyhow, particularly in the case of tumour involvement of the bone marrow.

For evaluation of the clinical performance of $^{89}$Zr-Zevalin PET, a pilot imaging study was conducted in one patient with CD20+ B-cell NHL. Serial PET camera images showed selective uptake in all tumour lesions that had previously been identified by [$^{18}$F]FDG-PET. Evaluation of these $^{89}$Zr-PET images suggests that the favourable biodistribution of $^{89}$Zr-Zevalin would make such an NHL patient a suitable candidate for high-dose $^{90}$Y-Zevalin RIT.

The general aim of our forthcoming RIT study is to assess whether addition of high-dose $^{90}$Y-Zevalin RIT to standard conditioning regimens for AuSCT in the treatment of aggressive NHL is safe, tolerable and effective. Dose escalation will be performed at fixed dose steps (not individualised). The implementation of $^{89}$Zr-immuno-PET in these studies will reveal whether it is recommendable to exclude patients with an unfavourable biodistribution (e.g. no tumour targeting or >1,500 cGy to organs) from treatment with standardised high-dose RIT.

*Conclusion*

To enable clinical PET imaging of Zevalin, procedures were developed for stable and reproducible coupling of the long-lived positron emitter $^{89}$Zr. Similar in vitro stability and biodistribution in NHL-bearing nude mice suggest that $^{89}$Zr-Zevalin can be safely used for monitoring $^{90}$Y-Zevalin biodistribution in a clinical setting. A pilot PET imaging study with $^{89}$Zr-Zevalin in a patient with CD20+ B-cell NHL showed clear uptake of $^{89}$Zr-Zevalin in all previously known tumour deposits. PET with $^{89}$Zr-Zevalin seems attractive for the quantitative prediction of pharmacokinetics, biodistribution and dosimetry of $^{90}$Y-Zevalin in high-dose RIT.

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ORIGINAL ARTICLE

# Biodistribution, radiation dosimetry and scouting of $^{90}$Y-ibritumomab tiuxetan therapy in patients with relapsed B-cell non-Hodgkin's lymphoma using $^{89}$Zr-ibritumomab tiuxetan and PET

**Saiyada N. F. Rizvi · Otto J. Visser · Maria J. W. D. Vosjan · Arthur van Lingen · Otto S. Hoekstra · Josée M. Zijlstra · Peter C. Huijgens · Guus A. M. S. van Dongen · Mark Lubberink**

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**Abstract**
*Purpose* Positron emission tomography (PET) with $^{89}$Zr-ibritumomab tiuxetan can be used to monitor biodistribution of $^{90}$Y-ibritumomab tiuxetan as shown in mice. The aim of this study was to assess biodistribution and radiation dosimetry of $^{90}$Y-ibritumomab tiuxetan in humans on the basis of $^{89}$Zr-ibritumomab tiuxetan imaging, to evaluate whether co-injection of a therapeutic amount of $^{90}$Y-ibritumomab tiuxetan influences biodistribution of $^{89}$Zr-ibritumomab tiuxetan and whether pre-therapy scout scans with $^{89}$Zr-ibritumomab tiuxetan can be used to predict biodistribution of $^{90}$Y-ibritumomab tiuxetan and the dose-limiting organ during therapy.
*Methods* Seven patients with relapsed B-cell non-Hodgkin's lymphoma scheduled for autologous stem cell transplantation underwent PET scans at 1, 72 and 144 h after injection of ~70 MBq $^{89}$Zr-ibritumomab tiuxetan and again 2 weeks later after co-injection of 15 MBq/kg or 30 MBq/kg $^{90}$Y-ibritumomab tiuxetan. Volumes of interest were drawn over liver, kidneys, lungs, spleen and tumours. Ibritumomab tiuxetan organ absorbed doses were calculated using OLINDA. Red marrow dosimetry was based on blood samples. Absorbed doses to tumours were calculated using exponential fits to the measured data.
*Results* The highest $^{90}$Y absorbed dose was observed in liver (3.2±1.8 mGy/MBq) and spleen (2.9±0.7 mGy/MBq) followed by kidneys and lungs. The red marrow dose was 0.52±0.04 mGy/MBq, and the effective dose was 0.87±0.14 mSv/MBq. Tumour absorbed doses ranged from 8.6 to 28.6 mGy/MBq. Correlation between predicted pre-therapy and therapy organ absorbed doses as based on $^{89}$Zr-ibritumomab tiuxetan images was high (Pearson correlation coefficient $r$=0.97). No significant difference between pre-therapy and therapy tumour absorbed doses was found, but correlation was lower ($r$=0.75).
*Conclusion* Biodistribution of $^{89}$Zr-ibritumomab tiuxetan is not influenced by simultaneous therapy with $^{90}$Y-ibritumomab tiuxetan, and $^{89}$Zr-ibritumomab tiuxetan scout scans can thus be used to predict biodistribution and dose-limiting organ during therapy. Absorbed doses to spleen

Saiyada N.F. Rizvi and Otto J. Visser contributed equally to this work.

S. N. F. Rizvi (✉) · A. van Lingen · O. S. Hoekstra · M. Lubberink
Department of Nuclear Medicine and PET Research,
VU University Medical Center,
PO Box 7057, De Boelelaan 1117,
1007 MB Amsterdam, The Netherlands
e-mail: snf.rizvi@vumc.nl

O. J. Visser · J. M. Zijlstra · P. C. Huijgens
Department of Haematology, VU University Medical Center,
De Boelelaan 1117,
1007 MB Amsterdam, The Netherlands

M. J. W. D. Vosjan · G. A. M. S. van Dongen
Department of Otolaryngology and Head and Neck Surgery,
VU University Medical Center,
De Boelelaan 1117,
1007 MB Amsterdam, The Netherlands

M. Lubberink
Department of Nuclear Medicine & PET, Uppsala University,
and Medical Physics, Uppsala University Hospital,
751 85 Uppsala, Sweden

were lower than those previously estimated using $^{111}$In-ibritumomab tiuxetan. The dose-limiting organ in patients undergoing stem cell transplantation is the liver.

**Keywords** Immuno-PET · Molecular imaging · Radioimmunotherapy · Ibritumomab tiuxetan · $^{89}$Zr · $^{90}$Y · Dosimetry · Lymphoma

## Introduction

Non-Hodgkin's lymphomas (NHL) account for 3% of all cancers worldwide [1]. Not all patients are cured with standard induction treatment [2]. For patients with aggressive, relapsed or progressive disease after first-line (immuno-) chemotherapy, a second-line regimen consisting of reinduction chemotherapy and consolidation with high-dose chemotherapy (carmustine, etoposide, cytarabine, melphalan; BEAM) followed by autologous stem cell transplantation (auSCT) is standard therapy with curative intent. A significant number of these patients will not be able to meet response criteria before transplantation or will relapse after transplantation [3]. The addition of anti-CD20 monoclonal antibodies (mAb) has substantially improved response rates and overall survival [4–6]. The $^{90}$Y-labelled anti-CD20 mAb ibritumomab tiuxetan (Zevalin®) is approved for treatment of patients with relapsed and refractory NHL. In recent studies, radioimmunotherapy (RIT) was integrated into upfront treatment in indolent NHL [7] or added to high-dose chemotherapy followed by auSCT, showing significant benefit [8, 9]. In standard treatment, the maximum amount of $^{90}$Y-ibritumomab tiuxetan given to patients is 15 MBq/kg with a maximum of 1.2 GBq [10]. The dose-limiting toxicity of RIT is myelosuppression. The effect of myelosuppression is prevented in auSCT with stem cell support. In general, higher external radiation doses are associated with improved clinical outcomes [11, 12]. To optimize therapeutic amounts of activity in individual patients, one needs to know the biodistribution of $^{90}$Y-ibritumomab tiuxetan and the effective dose to dose-limiting organs.

Biodistribution studies with $^{111}$In-ibritumomab tiuxetan and $^{131}$I-ibritumomab tiuxetan in humans have been published [e.g. 13, 14]. These studies were performed using gamma cameras, which complicates quantification. Labelling of ibritumomab tiuxetan with the positron-emitting isotope $^{89}$Zr allows for better quantitative assessment of biodistribution of ibritumomab tiuxetan with positron emission tomography (PET). Perk et al. [15] used *N*-succinyl-desferal for coupling of $^{89}$Zr to ibritumomab tiuxetan and showed that $^{89}$Zr-ibritumomab tiuxetan has a nearly identical biodistribution compared with $^{90}$Y-ibritumomab tiuxetan in mice, which allows for assessment of biodistribution of $^{90}$Y-ibritumomab tiuxetan using $^{89}$Zr-ibritumomab tiuxetan and PET. A scout scan procedure using $^{89}$Zr-ibritumomab tiuxetan to assess biodistribution of ibritumomab tiuxetan prior to therapy can then aid in selection of patients that can benefit from a potentially toxic therapeutic amount of $^{90}$Y-ibritumomab tiuxetan, as well as in optimizing the administered amount of $^{90}$Y-ibritumomab tiuxetan in the individual patient. However, even though $^{89}$Zr-ibritumomab tiuxetan is representative of $^{90}$Y-ibritumomab tiuxetan, a scout scan procedure with $^{89}$Zr-ibritumomab tiuxetan for this purpose is only valid when biodistribution during scout scan and therapy are similar, that is not dependent on the administered amount of labelled antibody.

The aim of this clinical, prospective study was to assess biodistribution and radiation dosimetry of $^{90}$Y-ibritumomab tiuxetan in humans using $^{89}$Zr-ibritumomab tiuxetan and to investigate whether pre-therapy scout scans with $^{89}$Zr-ibritumomab tiuxetan can be used to predict biodistribution during therapy, that is whether a co-injection of a therapeutic amount of $^{90}$Y-ibritumomab tiuxetan influences biodistribution of $^{89}$Zr-ibritumomab tiuxetan. A secondary aim was to determine the dose-limiting organ for therapy with $^{90}$Y-ibritumomab tiuxetan, in order to enable dose escalation and/or optimization with $^{90}$Y-ibritumomab tiuxetan in patients undergoing auSCT.

## Materials and methods

### Patients

Seven patients with relapsed or refractory aggressive B-cell (CD20-positive) NHL, who did not qualify for standard auSCT and were younger than 66, were included. All patients had previously been treated in first line with R-CHOP (cyclophosphamide, doxorubicin hydrochloride, vincristine and prednisolone, combined with rituximab). Second-line chemotherapy consisted of R-DHAP (cisplatin, cytarabine, dexamethasone combined with rituximab), R-VIM (etoposide, ifosfamide, methotrexate, combined with rituximab) and R-DHAP, which did not lead to at least partial remission as monitored by $^{18}$F-fluorodeoxyglucose (FDG) PET.

All patients had a WHO performance status of 0–2. Exclusion criteria were history of intolerance to exogenous protein administration, severe cardiac dysfunction [New York Heart Association (NYHA) functional classification class II–IV], severe pulmonary dysfunction (vital capacity or diffusion capacity<70%), unless clearly related to NHL involvement, hepatic dysfunction, bilirubin or transaminase≥2.5 times the upper normal limit, renal dysfunction (serum creatinine≥180 μmol/l or clearance≤40 ml/min), prior treatment with radiation therapy, uncontrolled infections, human immunodeficiency virus (HIV) positive and

NHL localization in the central nervous system. The study was approved by the Medical Ethics Committee of the VU University Medical Center and all patients signed a written informed consent prior to inclusion.

### Synthesis of $^{89}$Zr-ibritumomab tiuxetan and $^{90}$Y-ibritumomab tiuxetan

Ibritumomab tiuxetan was obtained from Bayer Schering AG (Berlin, Germany). $^{90}$Y (18.5 GBq/ml, radioactive half-life $T_{1/2}$=64.1 h) was obtained from IBA (Louvain-la-Neuve, Belgium) and $^{90}$Y-ibritumomab tiuxetan was prepared according to instructions of the supplier. $^{89}$Zr (≥0.15 GBq nmol$^{-1}$, $T_{1/2}$=78.5 h) was produced in-house by a (p,n) reaction on natural $^{89}$Y and isolated with the use of a hydroxamate column [16]. Methods for radiolabelling were used as described previously [15]. $^{89}$Zr-ibritumomab tiuxetan conjugates were analysed by instant thin-layer chromatography (ITLC) for radiochemical purity, and by a cell binding assay for immunoreactivity, as described before [16]. Endotoxin levels were assessed by use of a limulus amebocyte lysate (LAL) test system licensed by the US Food and Drug Administration (FDA) according to the instructions provided by the supplier (Endosafe®-PTS, Charles River Laboratories, Wilmington, MA, USA). These procedures resulted in a sterile final product with endotoxin levels<5 EU/ml. The radiochemical purity was always >97% (mean 97.4±1.5%). The immunoreactive fraction of $^{89}$Zr-ibritumomab tiuxetan preparations ranged from 72 to 81% (mean 75.0±3.2%).

### Scan protocol

Patients received standard treatment with 250 mg/m$^2$ Rituxan 1 week before and on the same day prior to both $^{90}$Y- and/or $^{89}$Zr-ibritumomab tiuxetan administrations. They received approximately 68±11 MBq $^{89}$Zr-ibritumomab tiuxetan 31 days before stem cell transplantation (Table 1) followed by three PET scans at 1, 72 and 144 h post-injection (p.i.) covering skull base to femur. Blood samples were drawn at 5, 10, 30 min and 1, 2, 72 and 144 h p.i. for measurement of absolute radioactivity concentrations in whole blood and plasma. In four patients, this was followed 14 days later by 15 MBq/kg or 30 MBq/kg of $^{90}$Y-ibritumomab tiuxetan, each in two patients, with a co-injection of 69±6 MBq $^{89}$Zr-ibritumomab tiuxetan, again followed by the same PET and blood sampling protocol. The first two patients were treated in hospital; therefore, all urine during the first 72 h after injection was collected and radioactivity in urine was measured. Patients 3 to 7 were treated in our outpatient department, until high-dose chemotherapy started 7 days before stem cell transplantation.

PET scans were made on a dedicated full ring ECAT EXACT HR+ (CTI/Siemens, Knoxville, TN, USA) PET camera in 3-D acquisition mode. Whole-body scans consisted of 7-min emission and 3-min transmission scans per bed position. Data were normalized and corrected for randoms, scatter, attenuation and decay. Attenuation correction was based on a transmission scan with rotating $^{68}$Ge rod sources. Images were reconstructed to 128×128 pixels with dimensions 5.15×5.15×2.43 mm using an attenuation- and normalization-weighted ordered subsets expectation maximization (NAW-OSEM) algorithm with 2 iterations with 16 subsets, followed by post-smoothing of the reconstructed image using a 5-mm full-width at half-maximum Gaussian filter. This resulted in images with a spatial resolution of about 7 mm. Quantitative accuracy of $^{89}$Zr PET scans was previously confirmed using phantom studies and comparisons between PET-derived and sampled blood radioactivity concentrations, as well as between PET-derived and biopsy tumour radioactivity concentrations [17].

### Volume of interest definition

The activity, percentage injected dose (%ID) and standardized uptake value (SUV) normalized to body mass in all organs that could be distinguished from background (lung, liver, spleen and kidneys) were determined using the mean activity concentration in volumes of interest (VOI) drawn over the entire organs using software developed in-house [18]. The activity in tumours was defined by drawing a 50%

**Table 1** Patient characteristics

| No. | Sex | Age | Weight (kg) | $^{89}$Zr | $^{89}$Zr+$^{90}$Y | $^{89}$Zr scan 1 (MBq) | $^{89}$Zr scan 2 (MBq) | $^{90}$Y (MBq) |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 50 | 84 | 1, 72, 144 | 1, 72, 144 | 49.7 | 65.5 | 1,267.7 |
| 2 | F | 50 | 90 | 1, 72, 144 | 1, 72, 144 | 48.0 | 88.5 | 2,473.40 |
| 3 | M | 43 | 71 | 1, 72, 144 | 1, 72, 144 | 79.2 | 77.7 | 1,151.3 |
| 4 | M | 61 | 78 | 1, 72, 144 | 72, 144 | 74.7 | 64.8 | 2,378.8 |
| 5 | M | 46 | 82 | 1, 72, 144 | – | 68.4 | | |
| 6 | M | 64 | 76 | 72, 144 | – | 77.5 | | |
| 7 | F | 37 | 82 | 1, 72 | – | 75.8 | | |

isocontour VOI. VOIs were drawn separately on all PET scans available for each patient. VOIs were drawn independently over each acquired PET image.

### Dosimetry

For calculation of $^{90}$Y residence times and absorbed doses, organ and tumour radioactivity were corrected for $^{89}$Zr decay, i.e. multiplied by exp(ln(2)t/78.4), with t the time since injection and 78.4 the radioactive half-life of $^{89}$Zr in hours, and then uncorrected for decay applying the radioactive half-life of $^{90}$Y, i.e. multiplied by exp(−ln(2)t/64.1), with the 64.1 h half-life of $^{90}$Y. Residence times for $^{89}$Zr- and $^{90}$Y-labelled antibodies were calculated as the area under the curve of the organ time-activity data determined by piecewise exponential fitting and assuming only physical decay after the last measurement, divided by the amount of injected activity. Residence time for the red marrow was estimated by assuming a red marrow radioactivity concentration of 30% of the whole blood activity concentration [19]. The residence time of the remainder of the body was calculated as the maximum possible residence time [the radioactive half-life of the tracer divided by ln(2)] minus the sum of residence time of source organs including red marrow, assuming no excretion during the time course of the scans. Tumour absorbed doses were estimated assuming local deposition of all radiation emitted by $^{90}$Y. The area under the curve of the tumour time-activity curves was estimated using a single exponential fit to two measured data points at 72 and 144 h p.i. Absorbed doses were calculated using the OLINDA/EXM 1.0 software [20].

### Statistical analysis

Total $^{89}$Zr-ibritumomab tiuxetan uptake in organs and tumours during the scout procedure and during treatment were compared using regression analysis.

## Results

Patient characteristics are shown in Table 1. All patients tolerated $^{89}$Zr-ibritumomab tiuxetan well, with no adverse reactions noted. Three patients underwent all scans both prior to and during therapy. Two patients (1 and 3) received standard therapy activity of $^{90}$Y-ibritumomab tiuxetan and two patients (2 and 4) were treated with a higher $^{90}$Y-ibritumomab tiuxetan activity. Three patients (5–7) did not receive the co-injection of $^{89}$Zr- and $^{90}$Y-ibritumomab tiuxetan because of logistical problems. One patient (7) had to be excluded from further analysis because he did not undergo a third PET scan (144 h p.i.) because of scanner problems. In total, data from six patients could be used to assess biodistribution of $^{89}$Zr-ibritumomab tiuxetan at 72 and 144 h p.i. Calculation of residence times in the first three patients, both with and without the 1-h p.i. scans, revealed no significant differences due to exclusion of the first scan (Pearson correlation coefficient $r$=0.99, linear regression with and without 1-h p.i. data point). Therefore, dosimetry data of patients 4 and 6 were included in the analysis and data from four patients could be used to compare biodistribution of $^{89}$Zr-ibritumomab tiuxetan prior to and during therapy.

Images at 1 h p.i. showed mainly blood pool activity and activity in heart, liver, spleen, bone marrow and kidneys. No activity was seen at tumour sites. The images at 72 and 144 h p.i. showed increasing activity on tumour sites and decreasing activity in source organs (Fig. 1). Negligible amounts of activity (<1% of administered activity) were found in urine.

Figure 2 shows SUV as a function of time for all source organs. As shown in Fig. 3, a strong correlation between estimated absorbed doses in different organs with $^{89}$Zr-ibritumomab tiuxetan without $^{90}$Y-ibritumomab tiuxetan and $^{89}$Zr-ibritumomab tiuxetan with $^{90}$Y-ibritumomab tiuxetan was found ($r$=0.97). The highest mean $^{90}$Y absorbed dose as calculated from the $^{89}$Zr-ibritumomab tiuxetan biodistribution was seen in the liver with 3.2±1.8 mGy/MBq (range 1.8–6.6 mGy/MBq, Table 2). However, absorbed dose to the spleen (2.9±0.7 mGy/MBq, range 1.8–3.8 mGy/MBq) was,

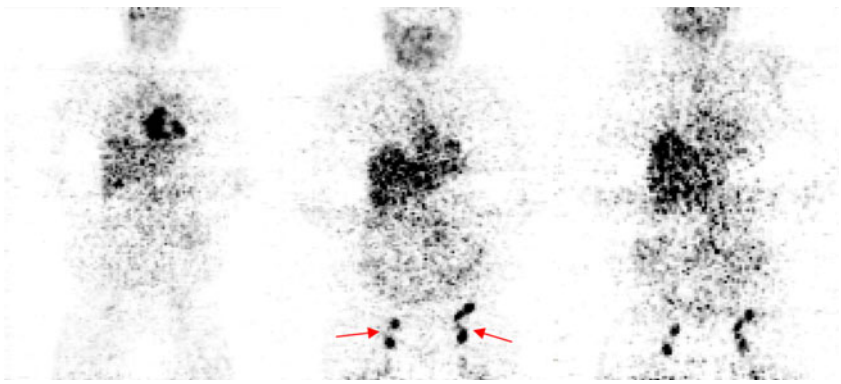

**Fig. 1** Typical coronal section $^{89}$Zr-ibritumomab tiuxetan images at 1, 72 and 144 h p.i. *Arrows* indicate tumour localizations

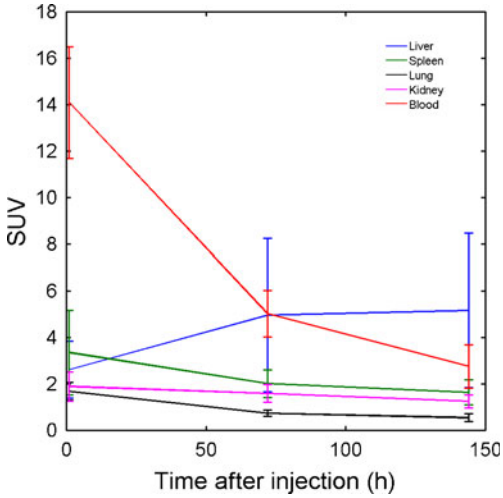


**Fig. 2** $^{89}$Zr-ibritumomab tiuxetan SUV versus time after injection in liver, spleen, lung, kidneys and blood (mean ± SD, $n$=6)

although marginally, higher in five of six patients. The effective dose was 0.87±0.14 mSv/MBq. Table 3 shows effective half-lives of $^{90}$Y-ibritumomab tiuxetan for the 1–72 and 72–144 h intervals. As expected, no effect of the higher treatment activity of $^{90}$Y-ibritumomab tiuxetan on the biodistribution in two of the four patients was seen, although this could not be tested statistically due to the small patient groups.

Tumour absorbed dose estimates were made for the 4 patients that underwent $^{89}$Zr-ibritumomab tiuxetan scans both prior to and during therapy with $^{90}$Y-ibritumomab tiuxetan, with 13 tumour localizations in total. The average size of the lesion VOIs was 3.3 cm$^3$ (range 0.8–11.3 cm$^3$). Tumour absorbed doses ranged from 8.6 to 28.6 mGy/MBq (mean 14.9 mGy/MBq). Figure 4 shows estimated tumour

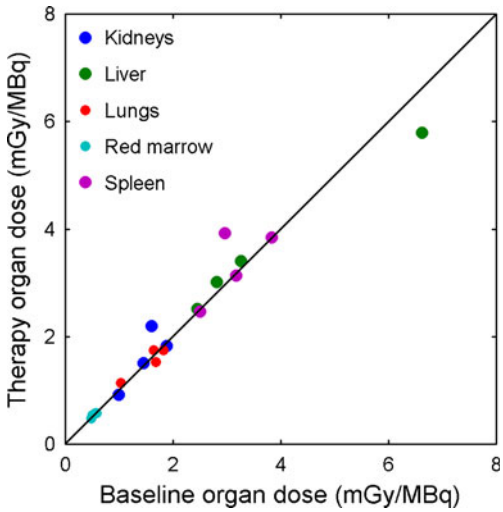


**Fig. 3** Absorbed organ doses estimated using a scout scan with $^{89}$Zr-ibritumomab tiuxetan prior to therapy versus those estimated using a simultaneous administration of $^{89}$Zr-ibritumomab tiuxetan and $^{90}$Y-ibritumomab tiuxetan. The *solid line* is the line of identity

absorbed doses. Correlation between absorbed doses estimated using a scout scan and during therapy was moderate ($r$=0.75), with no significant differences between baseline and therapy absorbed dose estimates ($p$=0.09, two-tailed paired $t$ test).

Figure 5 shows the correlation between SUV of $^{89}$Zr-ibritumomab tiuxetan and absorbed dose of $^{90}$Y-ibritumomab tiuxetan in the liver. Correlation was high for SUV at 72 h p.i. as well as 144 h p.i. (both $r$=0.99), with a nearly identical relationship between SUV and absorbed dose for both time points (slope 0.50 and 0.49 mGy·MBq$^{-1}$·SUV$^{-1}$; intercept 0.85 and 0.89 mGy/MBq, respectively).

## Discussion

To our knowledge, this is the first study describing the biodistribution and radiation dosimetry of $^{90}$Y-ibritumomab tiuxetan therapy as assessed by $^{89}$Zr-ibritumomab tiuxetan PET. All prior studies aiming to estimate $^{90}$Y-ibritumomab tiuxetan absorbed doses were performed using $^{111}$In-ibritumomab tiuxetan and single photon imaging. The advantage of using a positron-emitting isotope is that PET is inherently quantitative, whereas quantification with, especially planar, gamma camera imaging involves considerable uncertainties that can often surpass estimated activity concentrations themselves. Comparison of measured trues and random count rates as measured during scout scans and during scans with both $^{89}$Zr and therapeutic amounts of $^{90}$Y showed that the presence of $^{90}$Y had no effect on PET count rates and hence did not influence quantitative accuracy.

Although the results of the present work are generally consistent with previous studies using $^{111}$In-ibritumomab tiuxetan, there are a few important differences. The highest absorbed dose in the current work was found for the liver (3.2±1.8 mGy/MBq, range 1.5–6.6 mGy/MBq) and the spleen (2.9±0.7 mGy/MBq, range 1.8–3.6 mGy/MBq). Wiseman et al. [13], using $^{111}$In-ibritumomab tiuxetan in a study population of follicular lymphomas and transformed B-cell NHL, found the highest absorbed dose in the spleen with a median absorbed dose of 7.30 mGy/MBq with a range of 3.50–26.0 mGy/MBq followed by liver (4.60, range 2.20–11.0 mGy/MBq), lungs (1.90 mGy/MBq, range 1.30–4.30 mGy/MBq), red marrow (0.65 mGy/MBq, range 0.26–1.10 mGy/MBq) and kidneys (0.20 mGy/MBq, range (0.01–0.65 mGy/MBq). Although Fisher et al. [14], with a study population of low- and intermediate-grade NHL, found an absorbed dose to the spleen of 4.7±2.3 mGy/MBq, closer to the results in the current study, the spleen was the organ with the highest absorbed dose, followed by liver (3.6±1.4 mGy/MBq), red marrow (2.7±0.9 mGy/MBq), kidneys (2.4±0.6 mGy/MBq) and lungs (0.8±0.8 mGy/MBq). Shen et al. [21], using $^{111}$In-ibritumomab

**Table 2** $^{89}$Zr-ibritumomab tiuxetan and $^{90}$Y-ibritumomab tiuxetan organ residence times and absorbed doses as estimated using a scout scan with $^{89}$Zr-ibritumomab tiuxetan ($n$=6)

| | $^{89}$Zr-ibritumomab tiuxetan | | $^{90}$Y-ibritumomab tiuxetan | |
|---|---|---|---|---|
| | Residence time Mean ± SD (h) | Absorbed dose Mean ± SD (mGy/MBq) | Residence time Mean ± SD (h) | Absorbed dose Mean ± SD (range) (mGy/MBq) |
| Liver | 11.6±4.8 | 1.36±0.58 | 9.9±4.1 | 3.2±1.8 (1.5–6.6) |
| Spleen | 1.08±0.31 | 1.04±0.16 | 0.95±0.26 | 2.88±0.67 (1.83–3.83) |
| Kidney | 0.93±0.22 | 0.754±0.062 | 0.81±0.19 | 1.46±0.31 (0.99–1.88) |
| Lung | 2.83±0.55 | 0.63±0.11 | 2.54±0.48 | 1.47±0.34 (1.07–1.82) |
| Red marrow | 0.59±0.22 | 0.460±0.047 | 0.48±0.10 | 0.520±0.041 (0.485–0.581) |
| Remainder[a] | 96±5 | | 78±4 | |
| Effective dose (mSv/MBq) | | 0.55±0.07 | | 0.87±0.14 |

[a] Based on maximum residence time minus sum of source organ residence times

tiuxetan as well, also found the spleen to be the organ receiving the highest absorbed dose with a median absorbed dose of 6.1 mGy/MBq (range 1.8–17.8 mGy/MBq). Absorbed dose estimates from all publications referred to above and from the Zevalin package insert are summarized in Table 4. The doses from the Zevalin package insert were estimated with $^{111}$In-Zevalin. In this study, the spleen is the organ with the highest absorbed dose as well, at 9.4 mGy/MBq (range 1.8–20.0 mGy/MBq). Shen et al. (study population of follicular or transformed CD20-positive B-cell NHL) administered $^{111}$In-ibritumomab tiuxetan with and without additional treatment with rituximab and found no difference in absorbed radiation dose before and during treatment except in the spleen where a significant decrease of residence time was found. They also analysed the tumour absorbed dose which was consistent with our data with a median absorbed dose of 18.1 mGy/MBq (range 4.7–98.9 mGy/MBq). Shen et al. showed a decrease in residence time and volume of the spleen after long-term rituximab treatment compared to a single dose of rituximab before $^{111}$In/$^{90}$Y-ibritumomab tiuxetan. This could also apply to our study population and explain the difference from the four other studies where a single dose of rituximab was given prior to ibritumomab tiuxetan therapy. This observation is also in agreement with the study of Illidge et al. [22].

**Table 3** Mean (± SD) effective half-life of $^{90}$Y-ibritumomab tiuxetan as estimated using a scout scan with $^{89}$Zr-ibritumomab tiuxetan (1–72 h: $n$=5; 72–144 h: $n$=6)

| | Effective half-life (h) | |
|---|---|---|
| | 1–72 h p.i. | 72–144 h p.i. |
| Liver | 185±124 | 74±8 |
| Spleen | 43±15 | 54±6 |
| Kidney | 57±22 | 51±15 |
| Lung | 32±6 | 42±9 |
| Blood | 42±9 | 66±20 |

Differences in biodistribution between $^{111}$In-mAb and $^{90}$Y-mAb conjugates have been observed before in vivo in tumour-bearing mice and in cancer patients [23–25]. Perk et al. [26] showed in their study that biodistribution of $^{89}$Zr-Df-cetuximab and $^{88}$Y-DOTA-cetuximab ($^{88}$Y as a substitute for $^{90}$Y) is comparable for all observed organs, except differences in bone accumulation (sternum and thigh bone). What is more, the same was shown for $^{89}$Zr-ibritumomab tiuxetan and $^{90}$Y-ibritumomab tiuxetan in NHL-bearing mice [15]. Therefore, we can state that $^{89}$Zr-ibritumomab tiuxetan absorbed doses in liver and spleen are representative for $^{90}$Y-ibritumomab tiuxetan. Furthermore, the discrepancy between $^{111}$In-ibritumomab tiuxetan and $^{89}$Zr-ibritumomab tiuxetan uptake in liver and especially spleen has been described before when comparing $^{111}$In-DTPA-octreotide and $^{86}$Y-DOTA-octreotide as

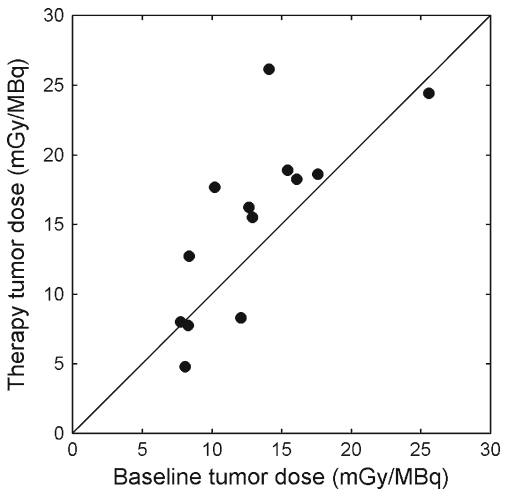


**Fig. 4** Absorbed tumour doses estimated using a scout scan with $^{89}$Zr-ibritumomab tiuxetan prior to therapy versus those estimated using a simultaneous administration of $^{89}$Zr-ibritumomab tiuxetan and $^{90}$Y-ibritumomab tiuxetan. The *solid line* is the line of identity

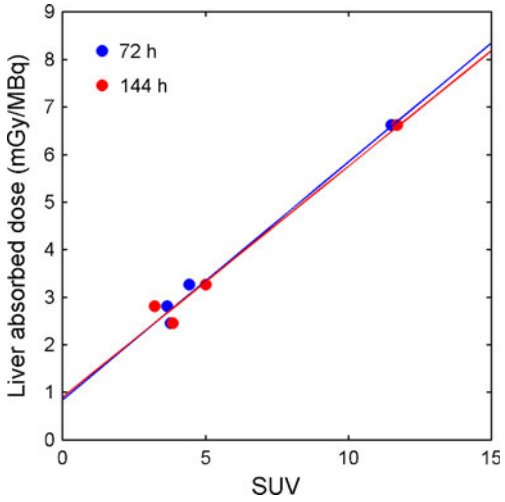


**Fig. 5** Absorbed $^{90}$Y dose to the liver, based on scout scans with $^{89}$Zr-ibritumomab tiuxetan, versus SUV of $^{89}$Zr-ibritumomab tiuxetan at 72 and 144 h p.i. The *solid lines* represent linear fits to the data

analogues of $^{90}$Y-DOTA-octreotide [27]. In this study $^{111}$In-DTPA-octreotide was shown to overestimate absorbed dose in kidneys and spleen, whereas absorbed dose to liver was underestimated in comparison to $^{86}$Y-DOTA-octreotide.

The effective dose found in the present study was generally higher than found in previous studies using $^{111}$In-ibritumomab tiuxetan (0.87±0.14 mSv/MBq compared to~ 0.5 mSv/MBq). The remainder of the body residence times were based on the assumption of no excretion of radioactivity, which is likely to lead to conservative effective dose estimates in the current study. Using whole-body VOIs instead, which in the present work would not include the legs, would have resulted in somewhat lower effective doses (mean 0.72 mSv/MBq). However, because the energy of the radiation emitted by $^{90}$Y is deposited within the same organ only, this does not affect $^{90}$Y-ibritumomab tiuxetan organ absorbed doses.

Red marrow and liver absorbed doses were 26 and 9% of the threshold for nonstochastic effects (2 and 35 Gy, respectively) [28]. Since the liver is the dose-limiting organ in patients undergoing auSCT, this implies that a considerably higher amount than 30 MBq/kg can be given to these patients without resulting in deterministic effects. Clinically, no significant abnormalities were seen and no significant decreases of liver function or liver function abnormalities were seen in this particular group of patients. Use of blood data to estimate marrow absorbed doses may underestimate dosimetry results if there is any interaction of the targeting agent with the bone marrow. However, this is not relevant for the patient group discussed in the present work, since patients will undergo auSCT following therapy.

Relative to organ dosimetry, tumour dosimetry shows a moderate correlation between baseline and therapy scans. This is most probably due to the small volumes of the tumours comparing the organ volumes, resulting in increased uncertainty in measurement of radioactivity concentrations. Tumour VOIs were drawn as 50% isocontours independently on each image, as recommended in the European Association of Nuclear Medicine procedure guidelines for tumour imaging with $^{18}$F-FDG [29]. The size of these isocontour VOIs is dependent on the statistically uncertain values for the maximum pixel, and variations in tumour VOI sizes over time result in variations in partial volume errors, which further affect the accuracy of absorbed dose estimates. To minimize these effects, tumour VOIs could be based on anatomical information (e.g. coregistered CT or MRI scans, preferably acquired using PET/CT or PET/MRI scanners), and partial volume corrections could be applied based on the anatomical information. Since the data in the present study were acquired on a stand-alone PET scanner, VOIs could not be drawn on coregistered CT images and no attempts to further improve the quantitative accuracy of tumour measurements were made. In addition, the limited number of data points and resulting uncertainty in tumour uptake, especially during the first 24–48 h after injection, may contribute to the low tumour dose correlation.

As Fig. 5 shows, SUV of $^{89}$Zr-ibritumomab tiuxetan at either 72 or 144 h p.i. may be a good predictor of the absorbed radiation dose to the liver during $^{90}$Y-ibritumomab tiuxetan therapy, although this should be confirmed in a larger study. Therefore, a single PET scan of the liver at 3 or 6 days after injection of $^{89}$Zr-ibritumomab tiuxetan could be sufficient to estimate the maximum amount of $^{90}$Y-ibritumomab tiuxetan that can be administered to an individual patient who will also undergo auSCT. This strategy will lead

**Table 4** Comparison of median absorbed doses of $^{90}$Y-ibritumomab tiuxetan for selected organs in mGy/MBq in different studies

| | Liver | Spleen | Kidney | Lung | Red marrow | Whole body (mSv/MBq) |
|---|---|---|---|---|---|---|
| Current study | 3.2 (1.5–6.6) | 2.9 (1.8–3.6) | 1.46 (0.99–1.88) | 1.47 (1.07–1.82) | 0.52 (0.49–0.58) | 0.87 (0.70–1.06) |
| Wiseman et al. [13] | 4.60 (2.20–11.0) | 7.30 (3.50–26.0) | 0.20 (<0.01–0.65) | 1.90 (1.30–4.30) | 0.65 (0.26–1.10) | 0.54 (0.46–0.78) |
| Fisher et al. [14] | 3.1 (2.3–6.6) | 4.3 (0.98–9.0) | 2.4 (1.4–3.9) | 0.60 (0.31–1.6) | 2.4 (1.7–4.5) | 0.55 (0.44–0.81) |
| Shen et al. [21] | 3.66 (2.11–11.62) | 6.14 (1.82–17.76) | 3.31 (1.95–4.65) | 1.10 (0.41–2.31) | 0.79 (0.32–1.22) | 0.48 (0.24–0.86) |
| $^{90}$Y-Zevalin® | 4.8 (2.9–8.1) | 9.4 (1.8–20.0) | 0.1 (0.0–0.3) | 2.0 (1.2–3.4) | 1.3 (0.6–1.8) | 0.5 (0.4–.07) |

to a therapy increasingly tailored to the individual patient. Although the present protocol requires administration of Rituxan prior to the pretreatment scan as well, this has limited influence on clinical feasibility since this is part of the routine treatment of the present patient group. Poor uptake in the tumour at the pre-therapy scan cannot be a reason to exclude patients from receiving this therapy. In tumour, not only the effect of RIT is important, but also the effect of Rituxan. Additional studies are needed for improved assessment of tumour dosimetry.

## Conclusion

Biodistribution of $^{89}$Zr-ibritumomab tiuxetan is not influenced by simultaneous therapy with $^{90}$Y-ibritumomab tiuxetan. Therefore, a pre-therapy scan with $^{89}$Zr-ibritumomab tiuxetan can be used to accurately predict radiation dosimetry during treatment with $^{90}$Y-ibritumomab tiuxetan. Absorbed doses to the spleen were lower than those previously estimated using $^{111}$In-ibritumomab tiuxetan. The dose-limiting organ in patients undergoing stem cell transplantation is the liver. In the future, a single $^{89}$Zr-ibritumomab tiuxetan PET scan may be sufficient to optimize the administered amount of $^{90}$Y-ibritumomab tiuxetan RIT in the individual patient when combined with auSCT.

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**Conflicts of interest** None.



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# No Harmful Impact of 90Yttrium-Ibritumomab Tiuxetan Combined with BEAM On Bone Marrow Microenvironment

Otto Visser, MD [1], Floortje Kessler [1], Lars Perk PhD [2], Maria Vosjan [2], Guus van Dongen PhD [2], Josée Zijlstra, MD, PhD [1], Peter Huijgens, MD, PhD [1], and Sonja Zweegman, MD, PhD [1].

[1] Department of Hematology, VU University Medical Center, Amsterdam, the Netherlands,

[2] Department of Otolaryngology-Head and Neck Surgery, VU University Medical Center, Amsterdam, the Netherlands.

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**Introduction.**

Recently, Yttrium-90 labeled anti-CD20 (90Y-ibritumomab tiuxetan, Zevalin®) has been introduced as a new therapeutic option in relapsed malignant B cell lymphoma. The results of adding 90Y-ibritumomab tiuxetan to high-dose BEAM with autologous stem-cell transplantation (auSCT) are promising. However, the toxic impact of radioimmunotherapy to the hematopoietic microenvironment, and its effects on stem cell homing and engraftment are largely unknown. Stromal Derived Factor-1 (SDF-1α) is a key regulator of stem cell engraftment. SDF-1α has been found to co-localize with hyaluronan (HA) on human bone marrow sinusoidal endothelium and endosteum, supporting transendothelial migration of human progenitor cells and their final anchorage within specific niches of the BM. External irradiation influences levels of SDF-1α and HA. Therefore, we studied the effect of 90Y-ibritumomab tiuxetan on in vivo SDF-1αand HA levels.

**Patients and methods.**

Patients with relapsed B cell NHL, treated with Zevalin-BEAM and autologous stem cell transplantation were included after obtaining their informed consent. At 3 different time points, bone marrow aspirates and peripheral blood samples of 9 consecutive patients were analyzed: day -22 (before Z-BEAM), day -8 (7 days after Zevalin (0.4 mCi/kg, max. 32 mCi (n=8) or 64 mCi (n=1) (before BEAM)) and day 0 (after BEAM and before auSCT). SDF-1α and HA protein levels were determined in bone marrow and peripheral blood plasma using ELISA. Also, SDF-1α mRNA expression was quantified by real time PCR. Quality of bone marrow stroma was determined by investigating CFU-F after 1 and 2 weeks and the percentage of confluency in cultures after 1, 2 and 3 weeks. Also, in 5 patients dosimetry of Zevalin was performed.

**Results.**

In 5 patients dosimetry of Zevalin was performed, by using Zirconium-89 labeled Zevalin as surrogate for PET scanning at day 0, +3 and +6 after injection. Patients received 3.4.10 * 6/kg(mean, range 1,9-8,3) CD34+ cells, and all but one showed engraftment. ANC>0.5 10*9/l was reached at 12,8 days (range 11-15, n=9), platelets>50 10*9/l at 18 days (range 11-38, n=8)), being comparable with retrospective data on BEAM transplantation. In the one patient not recovering, a second transplant did not result in platelet recovery. Zevalin alone did not affect bone marrow MNC count (23,3 vs 17,4 10*6/ml, p=0.40), CFU-F capacity (colonies > 50 cells: 2,4 vs 8,3, p=0.35) and stromal confluency (41,9 vs 62,1%, p=0.31). In addition, the levels of SDF-1α (4584 vs 5305 pg/ml, p=0.11) and HA (227 vs 247 mcg/ml, p=0.86) were not influenced. Following BEAM, production of SDF-1α (4584 vs 6166 pg/ml, p=0.01) and HA levels (227 vs 356 mcg/ml, p=0.03), significantly increased. A corresponding increase in SDF-1αmRNA copies was observed (0.16 vs 17.2 cps % GAPDH, p=0.02), indicating that induction of SDF-1αgene expression was involved. There was a trend in decreased quality of bone marrow stroma, as determined by MNC count, CFU-F capacity and confluency. Whereas Fibroblast Growth Factor increased the confluence of stromal culture before and after Zevalin, it didn't overcome the harmful effect of the BEAM chemotherapy.

**Conclusion.**

90Y-ibritumomab tiuxetan alone did not effect the bone marrow environment as measured by SDF-1α and HA. As expected, significant changes were found after high dose chemotherapy. Engraftment and repopulation after Z-BEAM and auSCT was similar to standard BEAM followed by auSCT.

# T-cel non-Hodgkin lymfoom en coeliakie: van refractair naar EATL.

O. Visser[1], A. Al-toma[2], C.M Mulder[3].

[1] Afdeling Hematologie, VUmc, Amsterdam
[2] Afdeling Maag, Darm en Leverziekten, St Antonius Ziekenhuis, Nieuwegein
[3] Afdeling Maag, Darm en Leverziekten, VUmc, Amsterdam

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**Refractaire coeliakie en de ontwikkeling van T-cel non-Hodgkin lymfoom in de darm: kliniek, diagnostiek en behandelingsmogelijkheden.**
O. Visser, A Al-toma. Oncollectie. 2007;1:2-8.

**Enteropathiegeassocieerd T-cellymfoom bij refractaire coeliakie: is dubbelbalon-enteroscopie de sleutel voor de diagnostiek?**
W.M. van der Veer, W.H.M. Verbeek, O.J. Visser, A. Al-Toma, C.J. Mulder, M.A.J.M. Jacobs.
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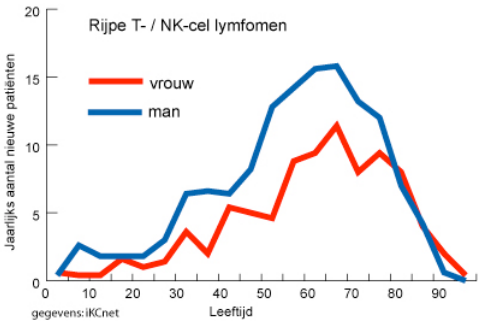
**T-cel non-Hodgkin lymfoom.**

*Inleiding.*

Ongeveer 12% van alle Non Hodgkin Lymfomen [NHL] zijn T-cel NHL of “Natural Killer” T-cel [NK/T-cel] NHL. T-cel NHL is een verzamelnaam van verschillende soorten en groepen lymfomen en deze NHL kunnen op iedere leeftijd ontstaan, maar de gemiddelde leeftijd is 60 jaar [figuur1].




[figuur 1]

*Jaarlijks aantal nieuwe patiënten per leeftijd met T-cel NHL in Nederland [bron: IKCnet]*

De indeling volgens de WHO classificatie geschiedt volgens de soort of het karakter van het NHL: leukemisch, cutaan [lokalisatie in de huid], nodaal [lokalisatie in een lymfklier] of extra-nodaal [tabel 1]. Naast het cutane T-cel NHL zijn de meest frequent voorkomende subtypes: perifere T-cel NHL [PTCL], perifere T cel lymfoom “unspecified” [PTLU], angioimmunoblastair T-cel NHL [AILT] en het anaplastisch T-cel NHL. Het EBV geassocieerde extra nodale NK/T-cel NHL van het nasale type is zeldzaam in de westerse samenleving en komt meer in Aziatische landen voor. In landen waar het HTLV-1 virus endemisch is, wordt vaker het “adult T-cell” leukemie/lymfoom gediagnosticeerd. Enteropathie geassocieerd T-cel NHL [EATL] is ook in Nederland een zeldzame diagnose, waarbij overigens de incidentie mogelijk lijkt toe te nemen, samenhangend met de toename van het aantal gediagnosticeerde patiënten met coeliakie. De schatting is dat jaarlijks ongeveer 10 patiënten met deze ziekte worden gediagnosticeerd.

[Tabel 1]

*Leukemic or disseminated*
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Aggressive NK-cell leukemia
- Adult T-cell leukemia/lymphoma

*Cutaneous*
- Mycosis Fungoides
- Sézary syndrome
- Primary cutaneous anaplastic large cell lymphoma
- Lymphomatoid papulosis [clinically not considered a neoplastic disorder]

*Other extranodal*
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-type T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T cell lymphoma

*Nodal*
- Peripheral T-cell lymphoma, unspecified [PTL, unspecified]
- Angioimmunoblastic T-cell lymphoma [AIL-T]
- Anaplastic large cell lymphoma [ALCL]

*Neoplasm of uncertain lineage and stage of differentiation*
- Blastic NK-cell lymphoma

***WHO histological classification of mature T-cell and NK-cell neoplasms.***
*[adapted from: Jaffe et al. WHO classification of tumours. Pathology and Genetics of Tumours of haematopoietic and lymphoid tissues. IARC Press: Lyon 2001, updated 2008]*

Het cutane T-cel NHL buiten beschouwing gelaten, heeft het T-cel NHL over het algemeen een slechtere prognose dan het B-cel NHL. Het anaplastisch lymfoom kinase [ALK] positieve anaplastisch T cel NHL [ALCL] vormt hierop een uitzondering: de prognose hiervan is beter. Het perifere T-cel NHL [PTCL] heeft een slechte prognose, enerzijds vanwege een kleinere respons op chemotherapie, anderzijds vanwege een grotere kans op recidief na bereiken van een remissie. Diverse studies hebben de prognostische waarde van de [voor de leeftijd gecorrigeerde] *International Prognostic Index* [aaIPI] die voor het agressief B-cel NHL is beschreven ook voor T-cel NHL aangetoond.

*Behandeling.*

Er bestaat geen consensus over de meest optimale behandeling van T-cel NHL. Alle subtypes worden meestal met hetzelfde schema behandeld. Een uitzondering vormt het extranodale NK/T-cel NHL van het nasale type, hierbij hebben verschillende studies een voordeel voor [lokoregionale] radiotherapie aangetoond al of niet in

combinatie met chemotherapie. Er zijn diverse studies verricht waarbij in eerste lijn een meer intensief chemotherapie schema is gegeven [toevoeging van ARA-C of hoge dosis CHOP gecombineerd met etoposide] maar in alle gevallen was het responspercentage en de overleving niet significant beter dan vergeleken met CHOP chemotherapie alleen. Vooralsnog worden de meeste patiënten in eerste lijn met CHOP chemotherapie behandeld.

Of autologe stamceltransplantatie [ASCT] in eerste lijn, na het bereiken van CR een rol speelt, is nog niet duidelijk. Een prospectieve fase 2 studie waarbij patiënten [n=62] met T-cel NHL in eerste lijn hoge dosis chemotherapie gevolgd door ASCT kregen, toonde aan dat 74% van de patiënten met het volledige schema behandeld konden worden. Zestien patiënten werden niet getransplanteerd omdat er sprake was van progressieve ziekte. Na een follow up van ruim 6 jaar bedroeg de totale overleving 34%. Hierbij was de prognose van patiënten met ALK+ anaplastisch T-cel NHL overigens beduidend beter.
Er zijn weinig data beschikbaar over allogeen transplanteren bij T-cel NHL. Er zijn enkele studies gepubliceerd waarbij langdurige overleving wordt gezien, en waarbij ook een effect van donor lymfocyten infusie wordt aangetoond. Dit pleit voor het bestaan van een zogenaamd “graft versus lymfoom” effect.

Conventionele chemotherapie met of zonder transplantatie heeft tot nu toe niet geleid tot spectaculaire verbeteringen in de overleving van patiënten met T cel NHL. Specifieke anti T cel middelen, zoals purine analoga [cladribine, fludarabine] hebben effect getoond in patiënten met cutaan T-cel NHL en PTCL.
Alemtuzumab is een monoclonaal antilichaam dat is gericht tegen het antigeen CD52, dat op veel, maar niet op alle T lymfocyten voorkomt. Alemtuzumab gecombineerd met CHOP chemotherapie is onderzocht bij 20 patiënten met een perifeer T-cel NHL. Hoewel deze combinatiebehandeling een goede respons toonde [90%], was de gemiddelde overleving beperkt [27 maanden] en kregen veel patiënten recidief NHL. Opvallend was het aantal patiënten met complicaties van de behandeling: koorts na chemotherapie [40%] en virus-reactivatie [CMV-reactivatie [35%], EBV gerelateerd NHL [15%]].
Bij de behandeling van T-cel NHL worden ook nieuwe middelen onderzocht. Pralatrexate, een afgeleide van methotrexaat, heeft in een aantal patiënten met T-cel

NHL opmerkelijke responsen laten zien, in sommige gevallen ook langdurig. Ook zijn er studies gedaan met denileukin diftitox [Ontak ®]. Dit is een fusie-eiwit dat het receptor bindende domein van interleukine 2 en het difterie toxine combineert.
Een nieuw onderzochte groep middelen in de behandeling van T-cel NHL zijn de zogenaamde histon deacetylase remmers [HDAC-i, bijvoorbeeld belinostat en romidepsin]. De eerste studies zijn vooral gedaan bij patiënten met een cutaan T-cel NHL en tonen vooralsnog een beperkte en voornamelijk kortdurende respons.
Recent is een fase II studie verschenen waarbij een grotere groep patiënten [n=130] met een perifeer T-cel NHL zijn behandeld met romidepsin. Ondanks dat patiënten eerder meerdere behandelingen hadden ondergaan, was er bij 25% van de patiënten een respons aantoonbaar, die gemiddeld 17 maanden duurde. Langere termijn resultaten en studies waarbij HDAC-i eerder in de behandeling wordt ingezet volgen.

*Conclusie.*
De prognose van het B-cel NHL is duidelijk verbeterd na de introductie van rituximab bij de behandeling. Door het ontbreken van dergelijke ontwikkelingen bij het T-cel NHL is de prognose hiervan vooralsnog slechter. Of alle soorten T-cel NHL op dezelfde manier moeten worden behandeld is niet duidelijk, uitzonderingen als het extranodale NK/T-cel NHL daargelaten. Met nieuwe kennis over genprofilering en nieuwe biologische parameters en met nieuwe middelen en strategieën kan de prognose in de toekomst hopelijk verbeterd worden.

**Coeliakie.**

*Inleiding.*

Coeliakie is een steeds vaker voorkomende ziekte die wordt veroorzaakt door een overgevoeligheid voor gluten, het voornaamste eiwit in bijvoorbeeld tarwe. De incidentie van coeliakie bij volwassenen varieert tussen 1.27 in Denemarken en 3.08 in Groot-Brittannië, oplopend tot 17.2 gevallen per 100,000 personenjaren in Finland. De incidentie van coeliakie bij alle leeftijden is in Nederland ongeveer 1.0 per 100,000.

Bij het eten van gluten ontwikkelt de patiënt een ontstekingsreactie in de darm waardoor de darmwand beschadigt, leidend tot buikklachten, diarree, resorptiestoornissen en gewichtsverlies. Een glutenvrij dieet is de enige remedie. Soms reageert de coeliakie patiënt niet op een glutenvrij dieet en persisteert de ontstekingsreactie in de darmwand, dit wordt refractaire coeliakie genoemd. Een ernstig gevolg van refractaire coeliakie is “enteropathy geassocieerd T-cel lymfoom” [EATL].

EATL wordt vrijwel uitsluitend gevonden bij patiënten bij wie op volwassen leeftijd coeliakie werd vastgesteld. Soms wordt ook pas nadat EATL is gevonden de diagnose coeliakie gesteld. Jaarlijks worden ongeveer 250 oudere patiënten [> 50jr] met coeliakie gediagnosticeerd en ongeveer 30 van deze patiënten ontwikkelen EATL. Het is niet te voorspellen welke patiënten dit betreft, wel wordt gezien dat deze groep in de periode voorafgaand aan EATL niet meer goed reageren op een glutenvrij dieet.

*Van coeliakie naar EATL.*

Coeliakie is een systeemziekte die kan optreden bij erfelijk gepredisponeerde mensen die na het eten van gluten histologische afwijkingen [vlokatrofie] van de dunne darm ontwikkelen. De vlokatrofie wordt histologisch ingedeeld volgens de zogenaamde Marsh criteria. Differentiaal diagnostisch moet onder andere giardiasis, tropische of collagene spruw, postinfectieuze diarree, koemelk intolerantie of de ziekte van Whipple overwogen worden.

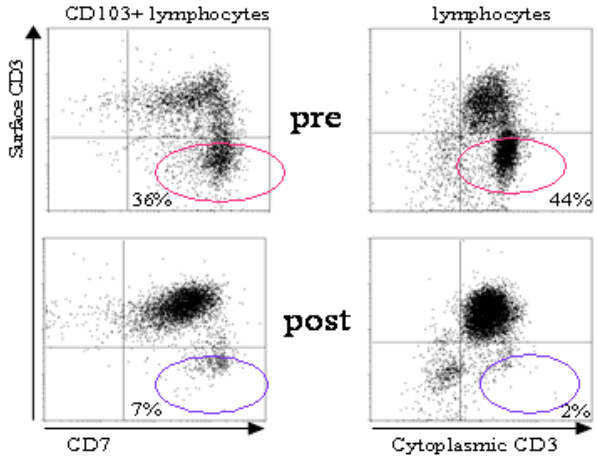
In de laatste jaren is er grote vooruitgang geboekt in het begrip van de ontstaanswijze van coeliakie. Het is duidelijk geworden dat inname van gluten bij coeliakie patiënten resulteert in een inadequate T-cel gemedieerde immuunrespons.

Daarnaast bestaat er een genetische aanleg: 95% van alle coeliakie patiënten heeft HLA-DQ2 [DQB1*02 en DQA1*05] en bijna 5% heeft HLA-DQ8 [DQB1*302 en DQA1*03].
Bij patiënten met coeliakie binden en presenteren HLA-DQ moleculen gluten-eiwitten aan specifieke T lymfocyten. Deze HLA-DQ-eiwit complexen veroorzaken een ontstekingsreactie in de dunne darm, hierdoor infiltreren lymfocyten in de lamina propria en neemt de hoeveelheid intra-epitheliale lymfocyten [IEL, lymfocyten in de darmwand] toe. Het gevolg is hyperplasie van de darmcrypten en atrofie van de darmvilli.
In een beperkt percentage [2-5%] van volwassen patiënten met coeliakie die zich strikt aan het glutenvrij dieet houden ontstaat een beeld van refractaire coeliakie ["*refractory celiac disease*", RCD]. Immunofenotypering van IEL onderscheidt twee groepen patiënten met RCD: een groep met normale IEL [RCD type I, RCDI] en een andere groep met aberrante IEL: onder andere ontbreekt hierbij expressie van oppervlakte CD3 en CD8 op de T lymfocyten [tabel 2]. Deze laatste groep patiënten [RCD type II, RCDII] laat zich moeilijk behandelen: azathioprine/prednisolon, ciclosporine of interleukine-10 behandeling heeft geen resultaat. Het lijkt er op dat patiënten met RCD vaker homozygoot voor DQ2 zijn [60%] dan patiënten met een gewone coeliakie [20%]. Patiënten met RCDI presenteren zich waarschijnlijk op een iets jongere leeftijd dan patiënten met RCDII.
De prognose van RCDI lijkt goed. Overlijden gerelateerd aan deze vorm van coeliakie of ontwikkeling van EATL werd niet waargenomen. Daarentegen bestaat er bij de groep patiënten met RCDII een sterk verhoogde kans op het ontwikkelen van EATL [60-80% binnen vijf jaar na diagnose].

Bij patiënten met RCD kunnen IEL geïsoleerd worden uit darmbiopten die vervolgens immunofenotypisch worden gekarakteriseerd. Diverse celoppervlakte kenmerken (CD) worden getest op een CD45 positieve populatie IEL: CD4, CD7, CD8, CD16/56, CD19, CD103 en TCR-γδ. Ook kan klonaliteitsanalyse verricht worden met behulp van T cel receptor-γ gen-herrangschikking testen waarbij eventuele monoklonaliteit aan worden aangetoond. Het onderscheid tussen RCD type I en II is vervolgens mede mogelijk op basis van immunofenotypische detectie van aberrante T cellen binnen de IEL populatie. Twee typen aberrante T cellen populaties worden gedefinieerd: het percentage CD7+CD3- van CD103+ IEL en het percentage CD3+

surfaceCD3- % van CD103+ IEL [figuur 2]. Het percentage aberrante T cellen zegt iets over de respons op behandeling en de mogelijke kans op ontwikkeling van EATL.

[Figuur 2]




*Twee typen aberrante T cellen populaties: het percentage CD7+CD3- van CD103+ IEL en het percentage CD3+ surfaceCD3- % van CD103+ IEL.*
*Voorbeeld bij een patient voor [pre] en na behandeling [post].*

Bij RCDII is het aantal IEL duidelijk toegenomen en juist uit deze cellen kan EATL ontstaan. Er bestaat overtuigend moleculair en immunofenotypisch bewijs dat aantoont dat een monoklonale maligne T-cel populatie kan ontstaat uit de IEL bij patiënten met RCD. Klonale expansie van deze monoklonale T cel populatie kan vervolgens leiden tot EATL. Hoewel de monoklonale IEL bij patiënten met RCDII als maligne beschouwd kunnen worden, zien ze er cytologisch normaal uit en vormen ze geen tumormassa zoals bij EATL gezien kan worden. De diagnose EATL is gebaseerd op darmbiopten, waarbij histologische en immunohistologische kenmerken wijzen op een anaplastisch T-cel lymfoom [CD3 cytoplasmatisch +, CD8-, CD30+]. Differentiaal diagnostisch moet een dunne darm carcinoom worden uitgesloten.
Samenvattend, patiënten met een gluten overgevoeligheid kunnen een spectrum aan ziektebeelden tonen: van asymptomatische coeliakie tot RCD met aberrante T lymfocyten, waarbij niet zelden een maligne T cel kloon tot de ontwikkeling van EATL kan leiden.

*Verschillende klinische stadia van coeliakie.*
De klinische presentatie van coeliakie is erg gevarieerd: gastrointestinale klachten zoals diarree, gewichtsverlies, groeiachterstand, braken, buikpijn, opgeblazen gevoel, anorexia en obstipatie kunnen voorkomen. De aanwezigheid van overgewicht of obesitas sluit de diagnose niet uit. Coeliakie kan zich ook zonder gastrointestinale klachten presenteren. Kenmerkende voorbeelden zijn dermatitis herpetiformis of anemie. Andere, meer zeldzame verschijnselen kunnen zijn: geringe lichaamslengte, recidiverende abortus, vermoeidheid, vitaminedeficiënties of recidiverende stomatitis. Coeliakie kan geassocieerd zijn met auto-immuunziekten zoals bepaalde schildklierziekten. Ook worden bij patiënten met coeliakie diverse neuropsychiatrische aandoeningen zoals depressie, angst, perifere neuropathie, ataxie, epilepsie en migraine gezien.

Bij coeliakie worden de volgende klinische stadia onderscheiden:

1. *Symptomatische coeliakie.*
Dit is de meest bekende vorm, waarbij patiënten met malabsorptie en buikklachten een volledig ontwikkelde gluten-geïnduceerde trias van vlokatrofie hebben. Deze trias toont IEL, crypthyperplasie en vlokatrofie. Daarnaast is er een vorm van coeliakie die gepaard gaat met extra-intestinale klachten zoals ijzergebreksanemie, groeivertraging, osteoporose of infertiliteit. Ook bij deze patiëntengroep wordt de trias van vlokatrofie gevonden maar vanwege het ontbreken van buikklachten wordt de diagnose coeliakie onvoldoende overwogen en kan er sprake zijn van een diagnostisch delay.

2. *Asymptomatische coeliakie.*
Dit betreft patiënten zonder klachten waarbij de diagnose coeliakie bij toeval wordt gesteld na serologisch onderzoek of nadat om een andere reden een endoscopie met biopsie is verricht.

3. *Latente coeliakie.*
Het betreft patiënten met een aangetoonde trias van vlokatrofie, die goed reageren op een glutenvrij dieet en bij herintroductie van gluten een normale darmmucosa

blijven behouden. Het kan ook patiënten betreffen met een normale darmmucosa die later in het leven alsnog een trias van vlokatrofie ontwikkelen.

4. *Refractaire coeliakie.*

Bij RCD responderen patiënten met de trias van vlokatrofie niet of niet langer op een glutenvrij dieet. De meest bekende reden voor falen van een glutenvrij dieet zijn al of niet opzettelijke dieetfouten. Bij RCD houden patiënten klachten en vlokatrofie, ondanks goede compliance voor het glutenvrije dieet. Ze kunnen complicaties als ulceratieve jejunitis of EATL ontwikkelen. Bij RCD wordt niet in alle gevallen een positieve coeliakieserologie gevonden. RCD wordt meestal gezien bij volwassenen en de gemiddelde leeftijd van diagnose is tussen de 50 en 60 jaar. De meeste van deze patiënten ontwikkelen ernstige malabsorptie met gewichtsverlies, buikpijn en diarree.

*Diagnostiek.*

De diagnostiek van coeliakie begint bij biopsie van de dunne darm: naast vlokatrofie is het aantal IEL verhoogd [> 30 per 100 epitheliale cellen]. De aanwezigheid van circulerende antistoffen zoals anti-endomysium [EMA] of anti-“tissue” transglutaminase [tTG] ondersteunen de diagnose. EMA-antistoffen kunnen jaren positief zijn en dienen als een secundair criterium gebruikt te worden. HLA onderzoek [HLA DQ2 en/of DQ8] heeft een ondersteunende waarde, vooral bij een twijfelachtige diagnose of om de diagnose uit te sluiten.

Indien vlokatrofie persisteert tijdens een glutenvrij dieet dient verdere diagnostiek plaats te vinden. Een beperkte compliance voor glutenvrij dieet is gerapporteerd tot 50% van de volwassen patiënten. Persisteren van verhoogde TTG-antistoffen of het opnieuw stijgen van de titer is hiervoor een belangrijke aanwijzing.

Na uitsluiting van dieetfouten dient RCD overwogen te worden. Het meest kenmerkende onderscheid tussen RCD type I en II bestaat uit een toename [15-25% van IEL] van het aantal aberrante T-lymfocyten [tabel 2]. Mucosale afwijkingen bij RCD dienen onderscheiden te worden van EATL. Ulceraties zonder EATL wordt ulceratieve jejunitis genoemd.

[Tabel 2]

| | **Diagnostische criteria voor RCD type I en II** |
|---|---|
| **RCD type I** | - Persisterende of recidief vlokatrofie ondanks glutenvrij dieet<br>- Minimaal vlokatrofie volgens Marsh III-a criterium<br>- Exclusie van andere oorzaken van vlokatrofie<br>- < 10% aberrante T cellen in bioptie<br>- IEL fenotype is normaal, CD3+, CD8+ en TCR+ |
| **RCD type II** | - als bij RCD type I, maar > 20% aberrante T cellen in biopsie<br>- IEL met normale cytologische kenmerken, maar aberrant fenotype ( normale expressie CD103 en CD7, verminderde expressie sCD3, toename intracytoplasmatisch CD3, en ontbreken van CD4, CD8 en TCR)<br>- Uitsluiten EATL (biopsie, radiologische diagnostiek) |

*IEL : intra epitheliale lymfocyten*
*CD: "cluster of differentiation"*
*EATL: "entropathy associated T cell lymphoma"*

Indien bij patiënten met RCDII een toename van gewichtsverlies, buikpijn, koorts en/of nachtzweten ontstaat, is verdere diagnostiek naar EATL van belang.
EATL wordt niet noodzakelijkerwijs vergezeld van vlokatrofie bij patiënten met een glutenvrij dieet. Als EATL wordt overwogen, wordt endoscopie en biopten van vrijwel de hele tractus digestivus geadviseerd: gastroduodenoscopie, dubbelballon endoscopie en visualisatie door middel van een video capsule endoscopie. Daarnaast moet de patiënt beoordeeld worden door de KNO-arts en moet een CT-scan van hals, thorax en buik verricht worden. In sommige gevallen kan het onderzoek worden uitgebreid met MRI-enteroclyse. Soms dient een laparotomie verricht te worden en waarbij zogenaamde "full thickness" biopten genomen kunnen worden. Aangetoond is dat een FDG-PET-scan bij sommige patiënten van nut kan zijn in het differentiëren tussen EATL en RCD.

*Behandeling.*
De behandeling van patiënten met RCD type I of II en EATL begint met goede supportive care. Intensieve begeleiding door gespecialiseerde diëtisten is noodzakelijk. Naast een glutenvrij dieet moet ook glutenvrije medicatie worden voorgeschreven, hiervoor bestaan diverse gespecialiseerde websites.

Patiënten met RCDI kunnen baat hebben bij een immuunsuppresieve behandeling, bijvoorbeeld met corticosteroïden of azathioprine. Ook kan een lokaal werkend corticosteroïd zoals budesonide worden overwogen. Er zijn case reports verschenen over behandeling met ciclosporine A, infliximab en tacrolimus, maar deze middelen dienen alleen overwogen te worden indien er te weinig respons op corticosteroïden bestaat of bij intolerantie voor azathioprine.
RCDII is meestal resistent voor behandeling. Respons op een behandeling met corticosteroïden sluit een onderliggende EATL niet uit. Naast een poging om de klinische conditie van de patiënt te verbeteren, is een ander doel van behandeling om de kans op het ontstaan van EATL te verkleinen. Behandeling met azathioprine is gerapporteerd, maar gaf ongunstige resultaten met een mortaliteit van 46%.
Behandeling met cladribine [2-chlorodeoxyadenosine, 2-CDA] heeft een plaats bij patiënten met RCDII. In een groep van 17 patiënten is het anti T-lymfocyten effect van dit synthetisch purine analoog aangetoond. De behandeling werd goed verdragen en had geen bijwerkingen. Zes patiënten [36%] hadden een goede klinische respons, en nog eens 6 patiënten hadden een significante daling van het percentage aberrante T-cellen. Eén patiënt vertoonde een complete klinische en immunologische respons, die tot 4 jaar na de behandeling aanhield. Het endoscopische beeld van ulceratieve jejunitis, een van de kenmerken van RCDII, verdween volledig bij 5 patiënten [29%]. Echter, 7 patiënten [41%] ontwikkelden een EATL, en overleden binnen 6-38 maanden na behandeling.
Inmiddels is ook onderzocht of meer intensieve chemotherapie met autologe stamcel transplantatie zinvol is voor deze patiëntengroep. Stamcel transplantatie is een behandelingsmogelijkheid die steeds vaker wordt toegepast bij patiënten met een auto-immuunziekte, die onvoldoende responderen op de standaard behandeling. Deze intensieve en experimentele behandeling is inmiddels met succes ingezet bij patiënten met multipele sclerose, reumatoïde arthritis, systemische sclerose, SLE en de ziekte van Crohn. Het concept van de behandeling in deze specifieke patiëntengroep is gebaseerd op immunoablatie door intensieve immunosupressie door hoge dosis chemotherapie, waarna naïeve T lymfocyten kunnen regeneren uit gereïnfundeerde stamcellen. De precieze plaats, indicatie en volgorde van behandeling van patiënten met RCDII met cladribine of met een autologe stamceltransplantatie, met een daarbij mogelijk kleinere kans op het ontwikkelen van EATL is nog niet duidelijk, en wordt verder onderzocht.

EATL heeft een slechte prognose, met een gemiddelde overleving van 31-39% en 11-20% na respectievelijk 1 en 5 jaar. Een prospectief onderzoek bij 35 patiënten met EATL die behandeld zijn met CHOP chemotherapie toonde een cumulatieve tweejaars overleving van 28% aan. Op dit moment zijn er verschillende studies gaande, waarbij bovenstaande en nieuwe behandelingsmodaliteiten worden onderzocht. Gezien de slechte prognose van patiënten met RCDII en zeker van patiënten met EATL zijn nieuwe behandelingsstrategieën noodzakelijk.

*Conclusie.*

Patiënten met een gluten overgevoeligheid kunnen een scala aan ziektebeelden ontwikkelen. Symptomatische coeliakie noopt tot een strikt glutenvrij dieet. Bij refractaire coeliakie kan met immunofenotypische technieken een aberrante T-cel kloon aangetoond worden. Deze kloon staat waarschijnlijk aan de basis van een maligne T-cel kloon die vervolgens tot de ontwikkeling van EATL kan leiden. De behandeling van EATL is moeilijk en nog steeds weinig succesvol. Daarom worden nieuwe strategieën ontwikkeld, om patiënten met RCDII te behandelen en zo het ontstaan van EATL mogelijk te voorkomen.

**Overzicht van hoofdstukken T1 – T5**

*Hoofdstuk T1*

In hoofdstuk T1 worden nieuwe inzichten in de behandeling van refractaire coeliakie besproken. Patiënten met RCDII hebben een aanmerkelijk verhoogd risico op ontwikkeling van EATL: 50-60% van de patiënten ontwikkelt deze ziekte binnen 4 tot 6 jaar. De uitdaging is om juist deze groep patiënten zorgvuldig te begeleiden en te behandelen om zo de ontwikkeling van EATL te voorkomen. In dit review wordt een overzicht gegeven van studies waarbij verschillende strategieën en behandelingen aan patiënten met RCDII zijn onderzocht, zoals behandeling met ciclosporine, azathioprine, budesonide, cladribine en hoge dosis chemotherapie met autologe stamceltransplantatie.

*Hoofdstuk T2*

In hoofdstuk T2 worden de eerste resultaten van autologe stamceltransplantatie bij patiënten met RCDII beschreven. Er zijn 13 patiënten met RCDII geëvalueerd, maar 6 van hen konden niet getransplanteerd worden [bij screening bleken afwijkingen aan het hart te bestaan [n=2], patiënten hadden inmiddels EATL ontwikkeld [n=3] en één patiënt had een te slechte lichamelijke conditie]. De overgebleven 7 patiënten [gemiddelde leeftijd 62 jaar] ondergingen een succesvolle verzameling van stamcellen [leukaferese]. Na conditionering met fludarabine en melfalan werd een autologe stamceltransplantatie uitgevoerd. Alle patiënten toonden een normaal herstel van de functie van het beenmerg. Er werden tijdens de behandeling geen belangrijke of onverwachte niet-hematologische bijwerkingen gezien. Er was geen transplantatie-gerelateerde mortaliteit. Bij meerdere controles van het darmslijmvlies werd nadien een significante afname van het aantal aberrante T-cellen gezien, met daarbij een aanzienlijke verbetering in het klinisch welzijn en normalisatie van hematologische en biochemische waarden [gemiddelde follow up was 16 maanden]. Deze eerste resultaten tonen aan dat hoge dosis chemotherapie en stamcel transplantatie haalbaar en veilig is in een geselecteerde groep patiënten met RCDII. Hopelijk kan op deze manier de ontwikkeling van EATL vertraagd of zelfs voorkomen worden.

*Hoofdstuk T3*

Een uitgebreid onderzoek met dezelfde strategie als in hoofdstuk T2 [autologe stamceltransplantatie] en met dezelfde categorie patiënten [RCDII] wordt in hoofdstuk T3 besproken. Gedurende 6 jaar zijn 18 patiënten met RCDII gevolgd en geëvalueerd omdat hun ziekte niet of onvoldoende reageerde op behandeling met cladribine. Uiteindelijk zijn 13 patiënten getransplanteerd en deze patiënten zijn minimaal 2 jaar gevolgd. Alle getransplanteerde patiënten hadden een aanzienlijke verbetering van hun klinische conditie en 5 patiënten hadden in biopten van het darmslijmvlies helemaal geen afwijkingen meer. Eén patiënt overleed ten gevolge van de transplantatie. De gemiddelde overleving na 4 jaar was 66%, dit in tegenstelling tot de 5 patiënten die om verschillende redenen niet getransplanteerd konden worden: van deze groep overleefde niemand [overlijden gemiddeld na 5 maanden]. In de groep van getransplanteerde patiënten ontwikkelde slechts 1 patiënt EATL, vier jaar na de behandeling.

Indien de diagnose EATL wordt gesteld, is de prognose slecht. De behandeling kan bestaan uit een operatie om de diagnose vast te stellen of er is een operatie noodzakelijk omdat er een acute perforatie van de darm is opgetreden. Daarna wordt de behandeling vervolgd met chemotherapie, in de meeste gevallen met CHOP-kuren. Gezien de prognose van EATL zijn meerdere strategieën ontwikkeld om het bereikte behandelingsresultaat na de CHOP behandelingen te bestendigen [consolideren] met een vorm van stamcel transplantatie. In hoofdstuk T4 wordt autologe en in hoofdstuk T5 wordt allogene stamcel transplantatie besproken.

*Hoofdstuk T4*

In hoofdstuk T4 wordt de behandeling van vier patiënten met EATL beschreven. Voor de behandeling met chemotherapie moesten drie van de vier patiënten geopereerd worden om de diagnose vast te stellen of omdat er een perforatie in de darm was opgetreden. Drie patiënten werden aansluitend aan de CHOP kuren getransplanteerd, één patiënt werd getransplanteerd nadat anderhalf jaar na de eerste behandeling een recidief EATL was vastgesteld. Ondanks deze intensieve behandeling overleden drie van de vier patiënten binnen enkele maanden na de transplantatie ten gevolge van opnieuw EATL. Het gebruikte chemotherapie- en het

conditionerings-schema zijn onvoldoende effectief. Nog intensievere schema's waarbij chemotherapie ook in het centraal zenuwstelsel kan komen, zijn noodzakelijk om de prognose van patiënten met EATL te verbeteren.

*Hoofdstuk T5*

In hoofdstuk T5 worden twee patiënten [61 en 66 jaar] met EATL beschreven die een allogene stamcel transplantatie hebben ondergaan. Na behandeling met CHOP kuren was bij beide patiënten geen ziekte meer aantoonbaar. Inmiddels was gebleken dat beiden tenminste één geschikte familiedonor [broer of zus] hadden die HLA identiek was. Aansluitend aan de eerste behandeling is een allogene stamcel transplantatie verricht, waarbij stamcellen van een HLA identiek familielid zijn gebruikt. Helaas werd na 6 respectievelijk 8 weken na de transplantatie bij beide patiënten opnieuw EATL vastgesteld waaraan beide patiënten zijn overleden. Onduidelijk blijft of eerder staken van afweer-onderdrukkende medicatie, om op die manier het zogenaamde "graft versus lymfoom" effect te introduceren, nuttig is.

EXPERT REVIEWS

# Novel approaches in the management of refractory celiac disease

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**Wieke HM Verbeek†, Marco WJ Schreurs, Otto J Visser, B Mary E von Blomberg, Abdulbaqi Al-Toma and Chris JJ Mulder**

*†Author for correspondence*
*VU University Medical Center, Department of Gastroenterology and Hepatology, PO Box 7057, 1007 MB, Amsterdam, The Netherlands*
*Tel.: +31 204 440 613*
*Fax: +31 204 405 54*
*whm.verbeek@vumc.nl*

Celiac disease is a gluten-sensitive enteropathy, which commits the patient to a life-long gluten-free diet. This is sufficient to treat the overwhelming majority of patients. However, a small group of these patients, mainly those diagnosed above 50 years of age, fails to improve histologically and clinically upon elimination of gluten from the diet. These patients are regarded as suffering from refractory celiac disease. In a subgroup of these patients a pre-malignant intraepithelial lymphocyte population can be detected in the small intestinal mucosa (type II). These patients are at a high risk of developing an enteropathy-associated T-cell lymphoma (50–60% within 4–6 years), which has a very poor prognosis and a 5-year survival of only 8%. The therapeutic challenge in these refractory celiac disease type II patients is targeting the aberrant intraepithelial lymphocytes to eventually prevent enteropathy-associated T-cell lymphoma development. Although management of these patients is difficult and therapeutic options are currently limited, novel treatment modalities are being explored.

**KEYWORDS:** 2-chlorodeoxyadenisine • aberrant T lymphocyte • antigen aberrancy • autologous stem cell transplantation • celiac disease • enteropathy-associated T-cell lymphoma • flow cytometry • immunophenotyping • intestinal γδ T cell • refractory celiac disease

Celiac disease (CD) is the most-common food intolerance in the general Western population, with a prevalence of 0.5–1% [1]. Ingestion of wheat gluten leads to chronic inflammation in genetically susceptible individuals, resulting in villous atrophy and flattening of the small intestinal mucosa. Prompt improvement of nutrient absorption and healing of the characteristic intestinal mucosal lesion is seen upon withdrawal of dietary gluten, which has to be excluded from the diet life-long.

In some patients, the intestinal damage can result in malnutrition and severe complications, but only 20–50% of the individuals that are affected have subjective symptoms [2]. CD has long been considered a gastrointestinal disorder of childhood with classical symptoms, but is now regarded as a chronic systemic autoimmune disease more often diagnosed in adults, in which the clinical picture can be very diverse [3].

The gluten-free diet (GFD) is usually sufficient to treat the overwhelming majority of CD patients and clinical improvement is usually evident within the first few weeks after commencing a GFD. However, in some adult patients it may take up to 2 years before a complete restoration of the intestinal mucosa is evident [4]. In a small percentage (2–5%) of adult-onset CD patients, serious complications develop in the form of refractoriness or development of (pre)malignant complications. They are regarded as suffering from refractory CD (RCD) when clinical and histological symptoms persist or recur after a former good response to a strict GFD, despite strict adherence to the diet for more than 12 months, unless earlier intervention is necessary [5–8]. RCD patients are nearly always adults 50 years of age and over. A relatively high percentage (52%) of these patients develops an enteropathy-associated T-cell lymphoma (EATL) within 4–6 years. EATL is the main cause of death in this patient group, which then has a 5-year survival of only 8% [9]. Early identification of these patients allows for early therapeutic intervention with a probable significant reduction in morbidity and mortality [10]. However, reliable identification of these patients remains difficult. Diagnostic criteria are depicted in TABLE 1.

According to the guidelines of the European Celiac Disease Working Group [11], RCD patients can be subdivided into RCD type I and type II patients, with phenotypically normal and

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aberrant intraepithelial T lymphocytes (IELs), respectively. IELs are considered aberrant when expressing cytoplasmic CD3ε but lacking surface expression of the T-cell markers CD3, CD4 and CD8 [12,13]. The presence of these IELs is directly associated with an increased significant risk of EATL development [6,9,14,15].

In this review, we aim to give an overview of RCD and its pathogenesis, establishing the diagnosis and the available therapeutic options.

## The pathogenesis & immunogenetics of (refractory) CD

### *Pathogenesis*

Celiac disease is an inflammatory condition caused by permanent intolerance for ingested wheat gluten and similar products in barley and rye. It is a multifactorial disease with an interplay between the triggering environmental factor, gluten, the main genetic risk factor, the *HLA-DQ2/8* genotypes and the autoantigen: the enzyme tissue transglutaminase (tTG) (Figure 1) [16]. Adaptive immunity, orchestrated by the lamina propria $CD4^+$ T cells, has a key role in the gluten-specific T-cell response. The gluten peptides that have passed the epithelial barrier and have been deamidated by tTG are presented by HLA class II molecules HLA-DQ2/8 on the cell surface of the antigen-presenting cells (APCs). Subsequently the deamidated gluten peptides are recognized by $CD4^+$ T cells and a proinflammatory T-cell response (Th1) is triggered.

In addition, other data suggest that certain parts of gluten (peptide 31–43/49) [17] may induce IL-15 production in the intestinal mucosa, which plays a key role in the gluten-induced innate part of the immune system and costimulates the aforementioned adaptive response. IELs are activated by IL-15, produced by epithelial cells and macrophages in response to the 'toxic' gluten, resulting in a cytotoxic effector response by these IELs by secretion of IFN-γ and expression of the innate immune receptor NKG2D (Figure 1). In response to IL-15 and stress, enterocytes upregulate MHC Class I chain-related A (MICA), the MHC-class-I-related epithelial ligand of NKG2D. Binding of this receptor–ligand pair can trigger cytotoxicity of IELs against the epithelial cells, independent of T-cell receptor (TCR) signaling. The interplay between innate and adaptive immunity against gluten orchestrates a progressive destruction of the epithelial layer and expansion of IELs, eventually resulting in villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis [18,19].

The question remains how RCD patients fail to regain intestinal homeostasis after gluten has been eliminated from the diet. In recent years, studies have pointed towards a key role of the latter innate immune response in this disease process, orchestrated by IL-15. This cytokine has a potent antiapoptotic effect and specifically induces the expansion and survival of the aberrant IELs that characterize RCD II. Furthermore, the potent proinflammatory effect of IL-15 triggers the secretion of IFN-γ by IELs and their cytotoxicity against epithelial cells, thereby promoting ongoing epithelial damage [20]. Uncontrolled overexpression of IL-15 may be the case in complicated CD, in which the highest amounts of IL-15 have been observed [21], possibly perpetuating epithelial damage and promoting the emergence of the aberrant T-cell population independent of gluten [20]. Also, in uncomplicated CD the

**Table 1. Diagnostic criteria of the different disease categories.**

| Disease category | Diagnostic criteria | Ref. |
|---|---|---|
| RCD I | Villous atrophy persisted or recurred despite strict adherence to a GFD (assessed by a dietician and negative TGA)<br>At least partial villous atrophy (Marsh IIIA) according to the modified Marsh criteria<br>Excluding other causes of villous atrophy<br>When ≤20% aberrant IELs in intestinal biopsy<br>IEL phenotype by flow cytometry is normal with the expression of surface CD3, CD(4/)8 and TCR | [6–9] |
| RCD II | Same as RCD I, in addition to the presence of ≥20% aberrant IELs in intestinal biopsy<br>The IEL have normal morphology, but exhibit an aberrant phenotype (lack of surface T-cell markers: CD3, CD4, CD8 and TCR; expression of surface CD7 and cytoplasmic CD3)<br>EATL has been excluded, as confidently as possible | [6–9] |
| UJ | Ulcerations in the jejunum with Marsh IIIA-C in noninvolved mucosa<br>Independent of the duration of the GFD and the percentage of aberrant IELs<br>EATL has been excluded, as confidently as possible | [60,67] |
| Secondary EATL | WHO classification of tumors of hematopoietic and lymphoid tissues<br>The patient is already known to have RCD | [9,59,60] |
| Primary EATL | WHO classification of tumors of hematopoietic and lymphoid tissues<br>No previous history of (complicated) celiac disease<br>Evidence of Marsh IIIA–C in noninvolved mucosa | [9,59,60] |

EATL: Enteropathy-associated T-cell lymphoma; GFD: Gluten-free diet; IEL: Intraepithelial lymphocyte; RCD: Refractory celiac disease; TCR: T-cell receptor, TGA: Anti-translutaminase antibodies; UJ: Ulcerative jejunitis.
Adapted from [9].

amount of IL-15 correlated with the degree of mucosal damage [21]. The level at which IL-15 production is deregulated in RCD II and EATL may be either transcriptionally or (post)translationally; however, this remains to be investigated in detail.

T-cell receptor $\gamma\delta^+$ IELs may also be involved in the pathogenetic mechanisms of RCD II and EATL, since we have found a relative deficiency of these cells in RCD II patients [22], possibly resulting in a lack of adequate anti-inflammatory TGF-β production with persisting epithelial damage and high IL-15 production. During gluten withdrawal and subsequent contraction of the TCR$\alpha\beta^+$ IEL response, TCR$\gamma\delta^+$ IELs may take on an anti-inflammatory role initiating mucosal repair. Evidence for this has recently been provided by Bhagat *et al.* who have shown that the human TCR$\gamma\delta^+$ IELs with the most potent regulatory potential increase upon commencement of a GFD in uncomplicated CD [23]. Compared with active CD, TCR$\gamma\delta^+$ IELs in treated CD showed increased capacity to suppress the cytotoxic arming of CD8$^+$TCR$\alpha\beta^+$ IELs via TGF-β production. Further studies are required to establish the exact functional role of TCR$\gamma\delta^+$ IEL in the pathogenesis of RCD and EATL development.

### *Immunogenetics*

Celiac disease is a multigenetic disorder, predominantly associated with the human leukocyte antigen (HLA) class II genotypes, which include *HLA-DQ2* (*HLA-DQA1*0501/HLA-DQB1*02*) and/or *HLA-DQ8* (*HLA-DQA1*0301/HLA-DQB1*0302*). Most CD patients (95%) carry the *DQ2* genotype, [24] encoded either in *cis* or in *trans,* and practically all remaining patients express *HLA-DQ8* [25]. These molecules represent MHC class II glycoproteins expressed on (professional) APCs (Figure 1). After deamidation of gluten peptides in the gut by the enzyme tTG, improving binding to HLA-DQ2/8 and subsequent presentation by APCs of these peptides to CD4$^+$ T cells, the T-cell response in the lamina propria of the small intestinal mucosa is initiated [26]. *HLA-DQ2* homozygous APCs putatively induce higher magnitude of gliadin-specific T-cell proliferation and cytokine secretion than *HLA-DQ2*/non-*DQ2* heterozygous APCs [27]. This may explain the strongly increased risk for CD development in *HLA-DQ2* homozygous individuals, [28] with an earlier onset and more severe disease manifestations [29,30]. In a recent study, we found a highly significant correlation between *HLA-DQ2* homozygosity and the development of serious complications of CD, in particular RCD II and EATL, implying a gene dose effect [31]. This would indicate that early diagnosis of CD and adherence to a GFD is particularly important for CD patients who are *HLA-DQ2* homozygous, as a strict GFD for more than 5 years reduces the risk for malignant complications to that of the general population [32].

The *HLA-DQ2/8* genotypes are necessary but not sufficient to develop the disease. Although almost all CD patients carry *HLA-DQ2/8*, so does approximately 40% of the healthy Western population [31,33]. Consequently, *HLA-DQ2/8* has a high negative predictive value for CD. In patients with (R)CD, the absence of *HLA-DQ2/8* is extremely rare. In case a patient is suspected for RCD and the *HLA-DQ2/8* genotypes are absent, the initial diagnosis of CD has to be doubted, since it virtually rules out RCD. Especially in case of negative serology before initiation of the GFD, the villous atrophy can probably be attributed to another cause (e.g., the presence of antienterocyte antibodies). Importantly, the mere presence of *HLA-DQ2/8* only supports the diagnosis, but does not rule out a different cause of villous atrophy, given its limited specificity for RCD.

*HLA-DQ2/8* is also a prerequisite for CD in people of heterogeneous ethnic backgrounds [33]. Regarding heritability, twin studies have shown a 75% concordance in monozygotic and

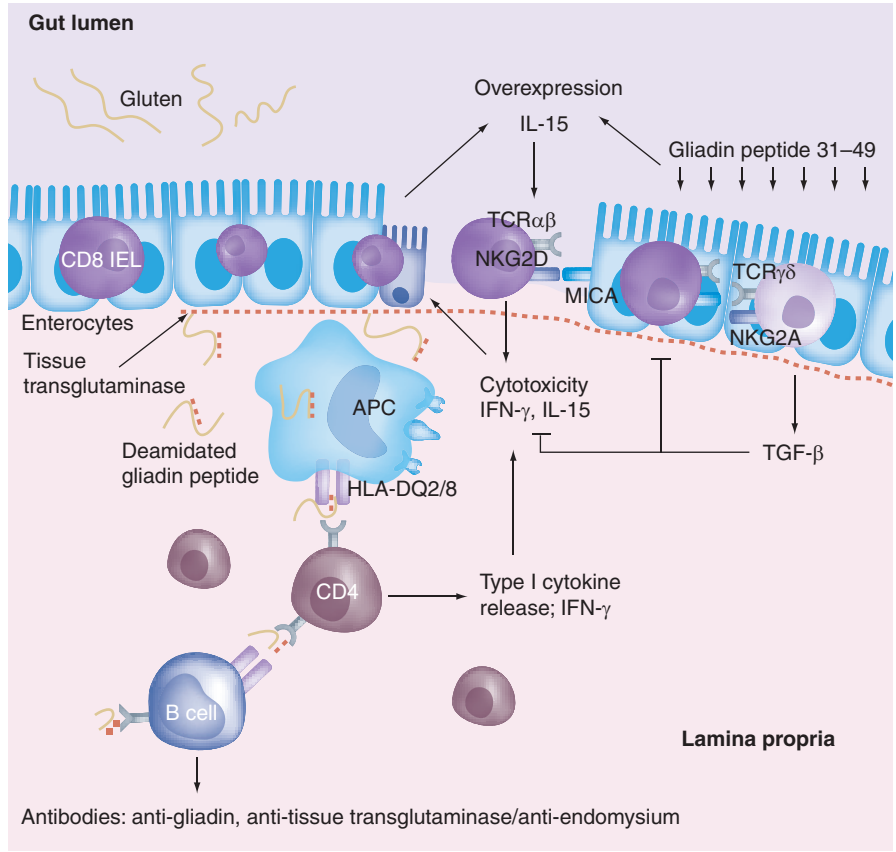


**Figure 1. Current view on the immunopathogenesis of celiac disease.**
APC: Antigen-presenting cell; IEL: Intraepithelial lymphocyte; HLA: Human leukocyte antigen; TCR: T-cell receptor.

11% concordance in dizygotic twins [34]. Furthermore, the sibling recurrence risk for CD in a British study was 10% [35]. These results imply that, although there is a stronger genetic component in CD than in many other complex diseases, the HLA genes contribute, at most, 40% of the heritable risk and thus HLA genes are not the only causative genetic factor for CD.

So far, the only non-HLA gene identified by positional cloning is the gene Myosin 9B (*MYO9B*) [36], although the effect of this gene could not be confirmed in CD populations in the UK, Spain, Italy and Scandinavia [37–40]. The limited replication of *MYO9B* association with CD in other populations reduced the validity of this gene and makes it difficult to interpret the role of *MYO9B* in CD pathogenesis. However, recently, positive associations were found between *MYO9B* gene polymorphisms and autoimmune diseases, including CD, in a Spanish cohort [41] as well as the *MYO9B* gene region, CD and dermatitis herpetiformis in a Finnish–Hungarian cohort [42]. In addition, we have recently shown that allelic variants of *MYO9B* also predispose to RCD II and EATL; one single nucleotide polymorphism (SNP) showed a significantly different allele distribution in RCD II and EATL patients compared with controls ($p = 0.00002$). A specific allele was significantly more frequent in RCD II and EATL patients than in CD patients ($p = 0.0003$). This *MYO9B* SNP predisposed to RCD II and EATL, and increased the risk for these complications in CD patients to a similar extent as and independent of *HLA-DQ2* homozygosity (odds ratio [OR]: 4.3 [95% confidence interval (CI): 1.9–9.8] and 5.4 [95% CI: 3.0–9.6], respectively) [43]. Furthermore, a recent genome-wide association study identified risk factors in the region harboring IL-2 and IL-21; genetic varation in this particular region was suggested to predispose for CD [44].

## Establishing the diagnosis of CD

### *Intraepithelial lymphocytes in CD*

Celiac disease is characterized by a permanent increase of TCR$\gamma\delta^+$ IELs with a concomitant elevation of infiltrating TCR$\alpha\beta^+$ IELs during the active stage of the process [45–47]. However, the TCR$\alpha\beta^+$ cells often decrease within months in response to gluten withdrawal, whereas for TCR$\gamma\delta^+$ cells this may take years to occur [47,48]. The contribution of TCR$\gamma\delta^+$ IELs to the pathogenesis of the villous atrophy in CD still remains unclear. Their persistent increase in CD patients who have recovered a normal mucosa upon a GFD suggests that TCR$\gamma\delta^+$ IELs do not induce the epithelial damage directly. This is supported by several studies that showed an increased number of TCR$\gamma\delta^+$ IELs in latent CD and dermatitis herpetiformis, in which the intestinal mucosa was still unaffected but progressed to villous atrophy eventually [49,50]. By contrast, the presence of TCR$\alpha\beta^+$ IELs correlates with the degree of villous atrophy in CD patients [47]. The increase in TCR$\gamma\delta^+$ IELs is considered the only permanent and highly sensitive and specific marker in uncomplicated CD, regardless of dietary treatment and mucosal morphology [45,46,51].

### *Histopathology of the duodenal biopsy specimen*

In CD, biopsy of the small intestine remains the gold standard for the diagnosis of CD. In CD, small bowel biopsy specimens show a characteristic, although not specific, mucosal lesion that impairs nutrient absorption by the involved bowel. Histopathological findings can be classified using the modified Marsh criteria for the gluten-sensitive spectrum [11,52,53]. The earliest lesion, classified as Marsh I, comprises lymphocytic enteritis with normal villous architecture and marked intraepithelial lymphocytosis (>30 lymphocytes per 100 enterocytes). A Marsh II lesion is present in case of intraepithelial lymphocytosis accompanied by crypt hyperplasia. The majority of CD patients are diagnosed with a Marsh III lesion, which consists of intraepithelial lymphocytosis, crypt hyperplasia and a moderate-to-severe reduction in villous height. This stage can be subdivided in Marsh IIIA with partial villous atrophy, Marsh IIIB with subtotal villous atrophy and Marsh IIIC with total villous atrophy.

### *Serologic screening for CD*

In a recent study, the anti-transglutaminase IgA antibodies (TGAs) and the antiendomysial IgA antibodies (EMA) proved to be the most sensitive serum antibody tests in a patient population referred for symptoms and signs of CD [33]. Furthermore, in a systematic review of the diagnostic performance of serologic tests for the diagnosis of CD, the pooled specificity of EMA was close to 100% and of TGA between 95 and 99% [54]. The overall sensitivities were approximately 90% but the titer correlated with the degree of mucosal damage. As a result, the sensitivity is lower (below 90%) in CD patients with a lesser degree of villous atrophy. The performance of the antigliadin antibodies was inferior to that of EMAs and TGAs, mainly as a result of their regular presence in healthy individuals and their regular absence in CD. An important pitfall in CD serology is the increased prevalence of IgA deficiency in these patients [55]. In order to avoid false-negative serology in such cases, simultaneous monitoring of either total (nephelometry) or specific (directed against i.e., *Escherichia coli*) IgA levels is required. In case of IgA deficiency, serologic screening for IgG antibodies against transglutaminase and preferentially also against endomysium should be performed. Although gliadin antibodies, if present at initial CD diagnosis, are well suited for monitoring the compliance to a GFD, TGA and/or EMA perform equally well.

## Establishing the diagnosis of RCD

### *Revision of the initial diagnosis of CD*

In a patient with persisting villous atrophy and CD-associated symptoms, refractory to a GFD, the first required step is to reassess the initial diagnosis of CD. The absence of *HLA-DQ2/8* genotypes or circulating EMAs/TGAs before the initiation of a GFD strongly suggests an alternative cause of villous atrophy (Figure 2, Box 1). Although not all CD patients have positive antibodies at presentation, serology tends to correlate with the degree of villous atrophy [56], and

in the case of total villous destruction, one usually finds positive antibodies. Furthermore, CD is characterized by increased IELs with a concomitant permanent elevation in TCRγδ$^+$ lymphocytes, which may also help to differentiate CD from other diseases [49].

### *Evaluating dietary compliance & assessing nutritional status*

The main cause for a lack of clinical and histological improvement after initiation of the GFD is dietary mistakes. Considering that a significant number of CD patients (~50%) suspected for RCD may indeed have inadvertent gluten ingestion, the dietary compliance has to be carefully reassessed by a skilled dietician and confirmed by negative serology for TGA (Figure 2) [57]. The latter usually reverts to negative within 3–6 months on a strict GFD and is strongly indicative for dietary mistakes, whereas, by contrast, EMA can persist for up to 1–2 years [4].

Importantly, the nutritional status of the RCD patients needs to be assessed by a dietician, as malnutrition is often present, requiring adequate nutritional intervention, adjusted to the needed energy and proteins. In case of intestinal failure, a nasogastric feeding tube or total parenteral nutrition might be indicated. To evaluate the intestinal energy-absorption capacity in RCD patients, the single fasting plasma citrulline concentration appears to have poor diagnostic accuracy [58]. Additional research on reliable tests reflecting enterocyte function is warranted to assess nutritional needs and to find biomarkers for permanent intestinal failure, intestinal adaptation and enterocyte repair.

**Box 1. Possible causes (other than celiac disease), for villous atrophy on duodenal biopsy specimens.**

*Villous atrophy with increased IELs*

- Giardiasis
- Postinfectious diarrhea
- Tropical sprue
- Collagenous sprue
- Protein intolerance (cow's milk, soya)

*Villous atrophy with (generally) normal number of IELs*

- Crohn's disease
- Tuberculosis
- Autoimmune enteropathy
- Radiation enteritis
- AIDS
- Common variable immunodeficiency syndrome
- Eosinophylic gastroenteritis
- Whipple's disease
- Immunoproliferative small intestinal disease

IEL: Intraepithelial lymphocyte.
Adapted with permission from [5].

### *Excluding other causes of villous atrophy*

The differential diagnosis of small intestinal villous atrophy is extensive (Box 1), although some are rare in Western countries, and includes Whipple's disease, Crohn's disease, tuberculosis, radiation enteritis, AIDS, common variable immunodeficiency syndrome, eosinophilic gastroenteritis, autoimmune enteropathy and immunoproliferative small intestinal disease. In the case of villous atrophy with increased numbers of IELs, other causes apart from CD may be: giardiasis, postinfectious diarrhea, tropical sprue, collagenous sprue and protein intolerance [5]. Before a patient can be regarded as a refractory celiac, these causes for a failure to improve histologically and clinically upon elimination of gluten from the diet must be reconsidered.

### *Flow cytometry of aberrant IELs as a prognostic marker for EATL development*

Intraepithelial lymphocytes with an aberrant immunophenotype are a prognostic parameter in RCD and their presence is associated with the development of EATL. Cellier *et al.* have first shown that RCD is associated with this abnormal subset of IELs of T-cell origin, expressing cytoplasmic CD3ε and restricted rearrangements of the TCRγ chain, but lacking surface expression of the T-cell markers CD3, CD4 and CD8 [13]. When normal expression of T-cell surface markers occurs (RCD I), the prognosis is less dismal than when an aberrant IEL population is present (RCD II), 50–60% of the latter patients develops EATL within 4–6 years [9]. These EATLs are thought to arise from the IEL compartment, with a phenotype comparable to the aberrant IELs in RCD II [59,60], due to a severe impairment in the intraepithelial homeostasis [16].

Considering the fact that RCD II patients are at a high risk for developing EATL, accurate discrimination between both types of RCD is of the utmost importance [9]. Diagnostic criteria are listed in Table 1 and the diagnostic work-up in Figure 2. We have recently defined a cut-off value of 20% aberrant IEL in order to discriminate between RCD I and II, using flow cytometry. IELs were isolated from intestinal biopsies and stained with fluorochrome-labeled monoclonal antibodies directed against CD3, CD4, CD8, CD7, CD45 and TCRγδ. Cytoplasmic staining of CD3 was performed after cell permeabilization. Flow cytometric analysis was performed on a standard four-color fluorescence activated cell scanner (FACSCalibur, BD Biosciences). The data were analyzed using the Cellquest software (BD Biosciences). Care was taken to analyze only viable cellular events based on light-scatter properties. All analyses were performed on lymphocytes, based on bright CD45 staining and low sideward scatter. Aberrant T cells were defined as CD7$^+$ cytoplasmic CD3$^+$, surface CD3, CD4 and CD8-negative cells. The 20% cut-off value has been established based on clinical observations, and via determination of reference ranges for aberrant T cells in the duodenal mucosa in different CD patient and control groups

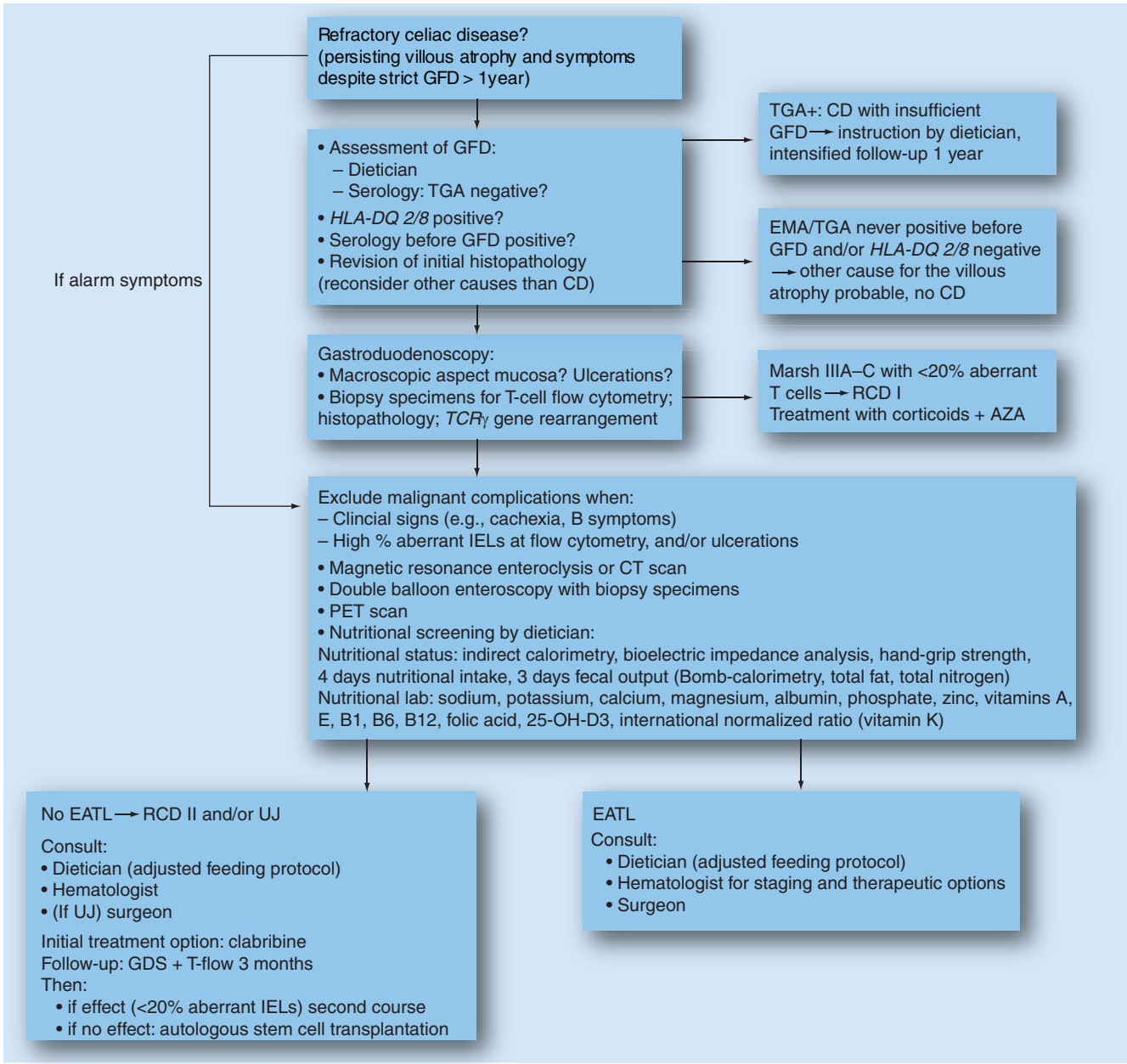


**Figure 2. Flowchart for the diagnostic work-up of a patient suspected for RCD or EATL at VU University Medical Center.**
CD: Celiac disease; EATL: Enteropathy-associated T-cell lymphoma; EMA: Antiendomysial antibody; GDS: gastroduodenoscopy; GFD: Gluten-free diet; HLA: Human leukocyte antigen; IEL: Intraepithelial T lymphocyte; RCD: Refractory celiac disease; TGA: Anti-transglutaminase antibody; UJ: Ulcerative jejunitis.

[9,22]. In 95% of non-RCD patients the highest percentage of aberrant IELs was 20%. Using this cut-off value, EATL development was exclusively seen in RCD II patients (median: 52% aberrant IELs; range: 27–94%). This cut-off value appeared reliable for early risk stratification [9] and targeted therapeutic options in RCD patients [10,61,62]. This is particularly important since, once overt lymphoma has developed, treatment outcome and survival are very poor [9,63]. In addition, quantification of aberrant T cells is useful for the subsequent follow-up of treated RCD II patients [10]. To estimate whether aberrant IELs are present in the small intestinal mucosa immunohistochemistry can be performed and a CD3:CD8 ratio can be determined, as described by Patey *et al.* [12]. However, in tertiary referral centers, the benefits of T-cell flow cytometric determination of aberrant IELs are the exact quantification (using a cytoplasmic CD3 staining) of use for follow-up. Prospective comparative studies between the two methods are currently lacking in the literature.

### *Clonality analysis in duodenal biopsy specimens as prognostic marker for EATL development*

Regarding T-cell clonality, we have recently compared the presence of aberrant IELs (using a cut-off value of 20%) with the frequently used (pre)malignant parameter, *TCR*γ gene rearrangement, for the predictive value of EATL development in a group of RCD patients [22]. Statistical analysis of our data revealed a much higher negative predictive value and sensitivity (both 100%) for aberrant IELs with regard to EATL development in RCD, when compared with clonality in a duodenal biopsy specimen (75 and 78%, respectively). The positive predictive values of these tests for EATL development in RCD were almost comparable (59% for aberrant IELs vs 50% for monoclonality). However, the majority of the RCD patients with phenotypically aberrant IELs had a monoclonal T-cell population, as shown in this study, as well as in other studies [6,13,60].

A study by Daum *et al.* showed similar results with regard to clonality: a clonal *TCR*γ-gene rearrangement could be found in the duodenal biopsy specimens of three out of eight patients with a resected EATL, twoout of two with ulcerative jejunitis (UJ), two out of three with RCD evolving to EATL and in one out of six RCD not evolving to EATL, whereas clonal *TCR*γ-gene rearrangements were present in all EATL specimens [64].

### *Excluding small intestinal malignancy*

Unexplained weight loss, abdominal pain, fever and night sweating should alarm physicians of an overt EATL. Other markers for overt EATL may be positive stool blood tests, increased lactate dehydrogenase or $\beta_2$-microglobulin. Patients with EATL can present in two different clinical ways (Table 1). There are patients with well-established CD who have responded to a GFD but then deteriorate because of the development of RCD II and/or (secondary) EATL. In the other group, patients develop EATL without a preceding history of complicated CD and these patients often present with perforation or obstruction (primary EATL) [9]. A high index of suspicion for an overt lymphoma should lead to an extensive work-up including upper and lower endoscopy, Ear–Nose–Throat-workup, CT or MRI scan of thorax and abdomen with enteroclysis, video-capsule enteroscopy (VCE) and double-balloon enteroscopy (DBE) in order to obtain histological specimens (Figure 2).

Enteroscopy using DBE or VCE should be performed in order to search for overt lymphoma. First described by Yamamoto *et al.* in 2001 [65], DBE is a new endoscopic technique with the potential to allow complete visualization of the entire small bowel and has been proved to be of value in patients with RCD [66]. The finding of mucosal ulcerations, mostly in the jejunum, defines the clinical picture of UJ but may also be present in EATL patients. Sometimes it is hard to differentiate between the two endoscopical features. UJ can be regarded as a 'cryptic' lymphoma [22,67].

New advances in small bowel imaging can improve the diagnostic accuracy in RCD and/or UJ patients and may be useful to exclude overt lymphoma, these include CT scan and magnetic resonance enteroclysis (MRE) [68,69]. Recently, van Weyenberg *et al.* have shown the latter to be a promising tool in discriminating RCD patients with a low and high chance of developing lymphoma, a four-point MRE-scoring system seemed able to identify those patients with (pre)malignant complications with acceptable sensitivity and specificity and a diagnostic accuracy of almost 90% [70]. This minimally invasive way to evaluate the total small bowel in patients with CD, without radiation exposure, could be of use in deciding which patients should undergo invasive diagnostic procedures such as DBE. Significant differences were observed in RCD II and EATL compared with responsive CD or RCD type I patients, regarding decreased jejunal folds (<10/5 cm), jejunoileal fold pattern reversal, bowel wall thickening and mesenterial fat infiltration. In addition, a splenic volume less than 120 $cm^3$ was associated with RCD II/EATL.

Furthermore, 18-fluorodeoxyglucose-PET scan has been investigated in a group of patients with EATL and RCD [71]. This modality has been able to visualize sites affected by EATL, as confirmed histopathologically, in prospective cohort of eight patients and 30 patients with RCD.

However, the exact diagnostic algorithm in RCD remains uncertain, as comparative studies between DBE, PET-CT or MRE and, for instance, conventional CT enteroclysis are missing in the literature. Future prospective comparative studies will have to point out the value of these techniques in the work-up of RCD and EATL.

## Therapeutic options in RCD

In the past decades, different therapies have been evaluated in RCD patients, including conventional corticosteroids, budesonide, infliximab, cyclosporine, azathioprine and IL-10, but there is no established treatment for these patients yet [61,72–78]. Reports claiming good responses are often difficult to interpret because of the absence of a clear distinction between RCD I and RCD II in these case reports and the small series of patients. All treatment modalities that have been evaluated in RCD are listed in Table 2.

A combination of prednisone and azathioprine is usually sufficient to treat RCD I patients without aberrant IELs [61,75]. Moreover, no CD-related mortality was observed in a cohort of treated RCD I patients studied, in which the overall 5-year survival was 96%, and no patients with RCD I developed RCD II or EATL within a mean follow-up of 5 years (range: 2–15 years) [9]. Cellier *et al.* also reported three RCD patients without aberrant T cells, who made a complete recovery with steroid therapy plus a GFD [6].

By contrast, all the aforementioned therapies are not successful in RCD II patients with high percentages of aberrant IELs. Results are unsatisfactory and progression to EATL usually occurs despite therapy, possibly even being accelerated by it [61]. This underscores the value of performing T-cell flow

**Table 2. Summary of the treatment modalities evaluated in refractory celiac disease.**

| Therapy | Type of report | Patients (n) | Type of RCD determined | Results | Ref. |
|---|---|---|---|---|---|
| Cyclosporine | Case report | 1 | ND | Remission | [72] |
| Cyclosporine | Open-label | 13 | ND | Clinical and histological improvement (61%) | [73] |
| IL-10 | Open-label | 10 | ND | Inconsistent response | [74] |
| Azathioprine | Open-label, prospective | 7 | Yes | Short-term clinical and histological improvement in five out of seven treated patients. Three died (leukopenic fever). Long-term follow-up. Evaluation: 46% mortality rate | [75] |
| Prednisone/ azathioprine | Open label | 10 RCD I, 8 RCD II | Yes | Seven out of eight RCD II died (six from EATL), ten out of ten RCD I with long-term survival | [61] |
| Budesonide | Open label | 4 RCD I, 3 RCD II | Yes | Good clinical and histological response in all RCD I patients, but not in two out of three of the RCD II patients | [76] |
| Infliximab | Case reports | 1 RCD I, 1 ND | Yes, ND | Remission, maintenance therapy (prednisone/azathioprine) | [77,78] |
| Alemtuzumab | Case reports | RCD II | Yes | Clinical improvement but persisting high percentages of aberrant IELs. One patient alive at 9-months follow-up, the others developed EATL and deceased | [81,82] |
| Pentostatine | Case report | 1 RCD II/UJ | Yes | Clinical and histological improvement with disappearance of ulcerations and aberrant IELs | [80] |
| Cladribine | Open-label, prospective | 17 RCD II | Yes | Clinical and histological improvement in 58%. No prevention of EATL development in the patients with persisting high percentages of aberrant IELs (41% died of EATL) | [62] |
| Autologous stem cell transplantation | Open-label, prospective | 7 RCD II | Yes | No major nonhematologic toxicity or transplantation-related mortality. Clinical and histological improvement with a significant reduction of aberrant IELs. One out of seven died 7 months after transplantation, Autopsy: chronic encephalitis, T lymphocyte infiltration (CMV, HSV negative). The other six in good clinical condition at 29-months follow-up (SD: 9.6; range: 19-43): One out of six persistent aberrant IELs with ulcerations. Three out of six Marsh 0, two out of six Marsh I-II. No EATL | [10] |

EATL: Enteropathy-associated T-cell lymphoma; IEL: Intraepithelial lymphocyte; ND: Not determined; RCD: Refractory celiac disease; SD: Standard deviation.
Adapted with permission from [62].

cytometry in these RCD patients, since the absence of aberrant IELs in small bowel biopsies at diagnosis of the refractory state seems to indicate a favorable prognosis and conventional treatment with prednisone/azathioprine is usually sufficient. Furthermore, quantification of aberrant IELs is useful for the subsequent follow-up of treated RCD II patients. The aim of therapy in RCD II is targeting of aberrant IEL to eventually prevent EATL development, as this T-cell population determines the risk for the development of EATL in RCD II and these patients are generally regarded as 'cryptic lymphoma' patients [6,9,14].

Cladribine (2-chlorodeoxyadenosine [2-CDA]), a synthetic purine nucleoside homologue with cytotoxic activity, is the only drug studied thus far showing a significant reduction in aberrant IELs in a large cohort of RCD II patients, although study results were still less than optimal [62]. Furthermore, the theoretical risk of accelerating lymphoma development has to be taken into account, as few cases of secondary malignancies after 2-CDA through T-cell immunodepression have been reported. It has proven valuable in the treatment of hairy cell leukemia [79], in which the pathological cells are also CD103 positive as are the aberrant IELs in RCD II. In our recent study, 17 RCD II patients were included and 2-CDA was given intravenously for 5 days (0.1 mg/kg/day), in 1–3 courses every 6 months, depending on the observed response. The drug has a relatively low systemic toxicity profile and was well tolerated by all patients, without serious adverse side effects. Cladribine therapy might be promising in stabilizing the patient's condition and improving the performance status and the histological picture, as was seen in 58% of the patients. However, it did not prevent eventual EATL development in all patients treated. Seven out of the 17 treated patients (41%) died from EATL. All seven patients had histological improvement of the mucosa, some even with normalization of the villous architecture. Although in some patients a significant decrease in aberrant IELs was observed, they still showed high percentages of these

cells after treatment (mean 69%; range 40–91%; standard deviation: 23). In six other patients who did not develop EATL, a significant decrease in aberrant IELs was observed, but only in two patients was the decrease below the 20% cut-off [22].

Most patients included in the aforementioned study were treated with prednisone and/or azathioprine for months before inclusion. Recently, we have started to treat RCD II patients upfront with cladribine without pretreatment with immunomodulatory drugs. Results so far are more promising, with normalization of the villous atrophy and a significant reduction in aberrant IELs below 20% in all four RCD II patients. Although follow-up is still limited, and it remains to be proven if EATL development can be delayed or even prevented in these patients, this approach appears to be more effective (Mulder C, Pers. Comm.).

Pentostatine is another purine analogue inducing T-cell depletion that is also commonly used and effective in hairy cell leukemia and chronic lymphoid leukemia. Only one RCD II/UJ patient treated with pentostatine and budesonide is known, tolerance was good and a dramatic clinical and histological improvement was observed, including not only villous regrowth in the jejunum (although not in the duodenum) but also disappearance of the aberrant IEL population [80]. These results seem very promising but larger series with a longer follow-up period would be needed to evaluate whether it is more effective than cladribine therapy and is able to prevent EATL development in these patients in the long run. However, unfortunately, since this drug is no longer available, such studies are not likely to take place.

Autologous hematopoietic stem cell transplantation (ASCT) is an increasingly accepted and effective treatment option for patients with severe autoimmune diseases refractory to conventional treatment and has been used successfully in patients with multiple sclerosis, rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus and Crohn's disease. The rationale for this strategy is based on the concept of immunoablation by intense immunosuppression using high-dose chemotherapy, with subsequent regeneration of the immune system from reinfused hematopoietic progenitor cells.

High-dose chemotherapy followed by ASCT after initial treatment with cladribine might be an alternative approach in these patients with a high risk for development of EATL. Our experience with ASCT in eight patients, after conditioning with fludarabine (40 mg/m$^2$/day orally) and melphalan (70 mg/m$^2$/day intravenously), is encouraging in improving the clinical condition but it remains to be established if development of EATL can be delayed or prevented, since follow-up is still limited (mean 15.5 months; range 7–30 months) [10]. No major nonhematologic toxicity or transplantation-related mortality was observed. In all but one patient, stem cells could be collected by leukapheresis, despite earlier treatment with cladribine. Importantly, there was a significant reduction in the aberrant IELs in duodenal biopsies, after ASCT, which was associated with restoration of villous architecture, although only in two patients the percentage of aberrant IELs was decreased below the 20% cut-off value eventually (the only two with 24-month follow-up values). Although these results are promising and this treatment seems feasible and safe for these patients we propose to adjust the protocol for future studies to have complete remission and eradication of the aberrant IEL clone before consolidation with ASCT.

Recently, in several patients, we were able to detect aberrant T-cell clone(s) in bone marrow, leukapheresis material and even in the peripheral blood, similar to the clone present in the small intestine (Visser O, Unpublished data). Therefore, because of the possible contamination of the graft by aberrant T cells, we aim to introduce T-cell depletion of the graft, using CD34 selection of the leukapheresis material. Furthermore, a more intensive conditioning regimen will be necessary trying to eradicate T cells before transplantation. A combination of fludarabine (intravenously) and alemtuzumab (anti-CD52) as conditioning regimens could be considered, followed by high-dose melphalan for myeloablation before ASCT.

Alemtuzumab (Campath-1H®) is a chimeric monoclonal antibody directed against CD52, a T- and B-cell marker. Three RCD II patients treated with this regimen have been described: they all improved clinically but only one was alive after 9-months follow-up (with 30% aberrant IELs left), while the other two patients developed EATL [81]. In one patient treated at our center in a very advanced stage of the disease, the aberrant IELs increased to 91% until EATL eventually developed [82]. A possible explanation may have been that the aberrant IELs were widely disseminated and were not sufficiently targeted by this single-agent antibody, given the fact that, in our patient, almost all aberrant T cells in the intestinal mucosa still expressed CD52, whereas in peripheral blood barely any (CD52$^+$) lymphocytes could be detected.

However, alemtuzumab has been demonstrated to have clinical activity in a number of T-cell lymphoproliferative disorders, such as T-prolymphocytic leukemia and cutaneous T-cell lymphoma, which are known for their chemoresistance and poor prognosis [83,84]. Single-agent therapy with alemtuzumab does not appear to be curative but alternative strategies, such as combinations with chemotherapy or consolidation of response with ASCT, may be promising [84]. Therefore, treatment of RCD II with alemtuzumab in combination with chemotherapy and/or ASCT should be explored as it may have additional value in combination with other regimens, to eradicate aberrant IELs in RCD II, before progression to overt lymphoma. Nonetheless, careful monitoring in specialist hematological centers is mandatory, as side effects of alemtuzumab can be tremendous, including high infectious and hematological toxicities. Furthermore, collaboration by experienced centers is needed in order to set up multicenter trials, pooling data of larger cohorts of these rare patients, to have more power to establish an appropriate treatment.

For future studies, anti-IL-15 may prove to be effective in the treatment of RCD II. Studies implicating a central role for IL-15 in the pathogenesis of RCD II, by orchestrating the cytotoxicity

of IELs against enterocytes, have suggested IL-15 as a promising therapeutic target in these patients [19–21]. As mentioned earlier, IL-15 is a cytokine with potent proinflammatory and anti-apoptotic properties, which is highly upregulated in the epithelium of patients with RCD II and EATL. In theory, blocking IL-15 might result in healing of the intestinal mucosa and elimination/apoptosis of the aberrant IEL, possibly preventing EATL development. Future trials with a humanized anti-IL-15 antibody in RCD II patients are to be awaited.

## Therapeutic options in EATL

Enteropathy-associated T-cell lymphoma is rare, except in the CD population, where the risk has been estimated to be as high as 19.2-times that of the general population [85]. The annual incidence rate of EATL has been reported to be 0.5–1 per million people in Western countries [86]. The outcome of EATL is very poor with current therapies, with 1- and 5-year survival rates in the range of 31–39% and 11–20%, respectively [9,87,88]. In a prospective multicenter study of 35 patients with EATL treated with six cycles of cyclophosphamide, doxorubicine, vincristine and prednisone (CHOP) chemotherapy, the cumulative 2-year survival was only 28% [88]. Chemotherapy for these patients can be complicated by small bowel perforation, gastrointestinal bleeding and development of enterocolic fistulae.

EATL patients often present at an older age (mean: >60 years of age) with advanced-stage disseminated disease, but if EATL is confined to part of the small intestine and if the affected segment (or segments) can be resected, the prognosis might be reasonable; some patients survive more than 5 years. Debulking by surgery might be mandatory, however, prospective studies are lacking in the current literature.

It seems that current chemotherapy and high-dose conditioning regimens followed by ASCT do not improve the survival in this type of aggressive lymphoma. Relapse regularly occurs within weeks to months after ASCT. Recently, we reported on the feasibility, safety and efficacy of ASCT in four EATL patients after undergoing cytoreductive therapy, including high-dose chemotherapy with or without partial small bowel resection [63]. The patients (two men, two women, mean age 65 years [range 60–69 years], all stage 4) received ASCT (three patients received upfront transplantation and one was transplanted only after relapse). After partial small bowel resection (three patients), induction chemotherapy and conditioning with BCNU, etoposide, Ara-C and melphalan (BEAM) chemotherapy ASCT was performed. Results showed that all four patients completed the mobilization and leukapheresis procedures successfully and subsequently received conditioning chemotherapy and transplantation. Engraftment occurred in all patients. No major nonhematological toxicity or transplantation-related mortality was observed. One patient had ongoing complete remission 32 months after transplantation but three patients died from progressive disease within a few months after ASCT [63]. It was concluded that ASCT for patients with EATL seems unsatisfactory. More encouraging results came from a recent report by Bishton *et al.* describing the treatment of six EATL patients, at an early stage of disease (40–59 years of age; stage 1–2), with ASCT after more aggressive chemotherapy, with two cycles of ifosphamide, etoposide and epirubicin, followed by two cycles of high-dose methotrexate (3 $g/m^2$) with folinic acid rescue and a BEAM [89]. Four patients remained alive in complete remission at 1.8–4.3 years and two relapsed.

Results of ASCT for EATL are, however, still unsatisfactory as patients often present at a more advanced stage of disease. Therefore, earlier diagnosis and the development of more stringent treatments, such as allogeneic stem cell transplantation with reduced intensity conditioning regimen are urgently required to improve the prospects of these patients, as this is considered a potentially curative treatment option for lymphoma patients in whom conventional treatment has failed [90]. Patients who undergo allogeneic SCT for non-Hodgkin's lymphoma, both indolent and high-grade types, have lower relapse rates than those who undergo ASCT [91–93]. Recently in our center, in an experimental setting the first patient with EATL has undergone ASCT with reduced intensity conditioning regimen.

Alemtuzumab, may also have a role in this setting, because studies have shown improvement in response rates and survival in treated patients with T-cell prolymphocytic leukemia and cutaneous T-cell lymphoma [83]. A recent study in patients with heavily pretreated peripheral T-cell lymphoma shows that alemtuzumab in combination with CHOP is a feasible chemoimmunotherapy regimen, effective in these patients with a high rate of complete remission achievement, and associated with mostly manageable infectious complications [94]. Therefore, treatment of EATL with alemtuzumab in combination with chemotherapy should be explored. At this time in The Netherlands, alemtuzumab in combination with CHOP chemotherapy is studied in this particular group of patients, and results are not available yet [101]. If patients are not suitable for allogeneic SCT, new options, such as a so-called 'sandwich-treatment' with cladribine, CHOP, cladribine (C,CHOP,C), could be considered.

In conclusion, it appears that current chemotherapy and high-dose conditioning regimens followed by ASCT do not improve the survival in this type of aggressive lymphoma. Relapse regularly occurs within weeks to months. Therefore, studies instituting therapy at an earlier stage, development of more effective treatments, including alemtuzumab, improving conditioning regimens and possibly the use of T-cell-depleted autologous grafts or allogeneic stem cell transplantation with reduced intensity conditioning regimen are urgently required to improve the prospects of these patients.

## Expert commentary & five-year view

The treatment of RCD patients remains a difficult problem, especially in case of the presence of an aberrant IEL population (RCD II). The RCD I patients respond well to

azathioprine and prednisone and do not seem to be at an increased risk for EATL or RCD II. However, currently there is no established treatment available for the RCD II and UJ patients to eventually prevent EATL, and therapeutic strategies are still evolving. Therapies directed at eradicating the aberrant IEL population, such as upfront cladribine, may be promising in combination with ASCT, as these were the only treatments which proved to significantly reduce number of aberrant IELs.

Single-agent therapy with alemtuzumab does not appear to be curative in RCD II; however, its use in combination with chemotherapy and ASCT may be promising. A further new approach for RCD II/UJ patients probably implies fine tuning the ASCT protocol by using T-cell-depleted grafts and optimizing preconditioning regimens. This dedicated approach will possibly have a significant impact on improving the dismal prognosis of these patients (which now have a 5-year survival of 58%). We think that multicenter collaboration and pooling of data would result in an effective treatment for these patients. These should be the main topics in the years to come.

Despite treatment with combination chemotherapy and high-dose conditioning regimens followed by ASCT, the survival of EATL remains very poor. Results of ASCT for EATL are unsatisfactory as patients often present with an advanced stage of disease and relapse occurs regularly. Therefore, instituting therapy at an earlier stage (if possible RCD II/UJ), development of more effective targeted treatments, including alemtuzumab, and improved conditioning regimens are needed. More importantly, the use of allogeneic SCT with reduced intensity conditioning may hold considerable promise in these EATL patients and should be further explored. If patients are not suitable for allogeneic SCT, new options, such as a so-called 'sandwich-treatment' with C,CHOP,C may have a role. To facilitate an optimal international collaboration, in order to set up multicenter trials for the treatment of RCD II/UJ and EATL patients, diagnostic criteria for these disease entities should be determined in a similar way to that done for CD in 2001. Hopefully this will be take place at the next *International Celiac Disease Symposium* in Amsterdam, The Netherlands, 6–8 April 2009.

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## Key issues

- In a patient with persisting villous atrophy and CD-associated symptoms, refractory to a gluten-free diet (GFD), the first required step is to reassess the initial diagnosis of CD and assess the compliance to the GFD. The absence of *HLA-DQ2/8* genotypes or the absence of circulating antiendomysial antibodies/anti-transglutaminase antibodies (TGA) before the initiation of a GFD strongly suggests an alternative cause of villous atrophy. The presence of TGA after more than 6 months on a GFD antibodies implies continued (inadvertent) gluten ingestion.
- When normal expression of T-cell surface markers is present in refractory CD patients (RCD I), the prognosis is better than when an aberrant intraepithelial lymphocyte (IEL) population is present (RCD II); 50–60% of the latter patients develop enteropathy associated T-cell lymphoma (EATL) within 4–6 years, after which the 5-year survival is only 8–20%.
- Quantification of aberrant IELs by flow cytometry is preferable to T-cell clonality analysis for identification of RCD patients at risk for EATL development. A cut-off value of 20% is of use in risk stratification, choice of therapeutic options and subsequent follow-up of RCD patients.
- A combination of prednisone and azathioprine is usually sufficient to treat RCD I patients, in which the overall 5-year survival is 96%, and in our study population, no patient with RCD I developed RCD II or EATL within a mean follow-up of 5 years.
- High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation after initial treatment with upfront cladribine may be an effective approach in the RCD II patients with the goal to eradicate the aberrant IEL population and eventually prevent EATL. This procedure is feasible without additional hematologic toxicity, but is only a therapeutic option in selected patients.
- To expand the horizon of the diagnostic and therapeutic arsenal in RCD II and EATL collaboration by experienced centers is needed in order to set up multicenter trials, pooling data of larger cohorts of these rare patients, to have more power to establish an appropriate treatment.

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## Website


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## Affiliations

- Wieke HM Verbeek
VU University Medical Center,
Department of Gastroenterology and Hepatology, PO Box 7057, 1007 MB,
Amsterdam, The Netherlands
Tel.: +31 204 440 613
Fax: +31 204 405 54
whm.verbeek@vumc.nl
- Chris JJ Mulder
VU University Medical Center,
Department of Gastroenterology and Hepatology, PO Box 7057, 1007 MB,
Amsterdam, The Netherlands
Tel.: +31 204 440 613
Fax: +31 204 405 54
cjmulder@vumc.nl
- Marco WJ Schreurs
VU University Medical Center,
Department of Pathology, Medical Immunological Laboratory,
De Boelelaan 1117, 1081 HV,
Amsterdam, The Netherlands
Tel.: +31 204 444 030
m.schreurs@vumc.nl
- B Mary E von Blomberg
VU University Medical Center,
Department of Pathology, Medical Immunological Laboratory,
De Boelelaan 1117, 1081 HV,
Amsterdam, The Netherlands
Tel.: +31 204 444 030
bme.vonblomberg@vumc.nl
- Abdulbaqi Al-Toma
St Antonius Hospital, Department of Gastroenterology and Hepatology,
PO Box 2500, 3430 EM, Nieuwegein,
The Netherlands
Tel.: +31 306 099 111
a.altoma@antonius.net
- Otto J Visser
VU University Medical Center,
Department of Hematology,
De Boelelaan 1117, 1081 HV
Amsterdam, The Netherlands
Tel.: +31 204 442 604
Fax: +31 204 442 601
o.visser@vumc.nl

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# Autologous hematopoietic stem cell transplantation in refractory celiac disease with aberrant T cells

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# Autologous hematopoietic stem cell transplantation in refractory celiac disease with aberrant T cells

Abdulbaqi Al-toma,[1] Otto J. Visser,[2] Hyacintha M. van Roessel,[2] B. Mary E. von Blomberg,[3] Wieke H. M. Verbeek,[1] Petra E. T. Scholten,[3] Gert J. Ossenkoppele,[2] Peter C. Huijgens,[2] and Chris J. J. Mulder[1]

Departments of [1]Gastroenterology, [2]Hematology, and [3]Clinical Pathology, VU University Medical Center, Amsterdam, The Netherlands

**Autologous hematopoietic stem cell transplantation (ASCT) is an increasingly accepted treatment for refractory autoimmune diseases. Refractory celiac disease with aberrant T cells (RCD type II) is unresponsive to available therapies and carries a high risk of transition into enteropathy associated T-cell lymphoma (EATL). This study reports on the feasibility, safety, and efficacy of ASCT in patients with RCD type II. Thirteen patients with RCD type II were evaluated. Seven patients (4 men, 3 women, mean age 61.5 years [range, 51-69 years]) underwent transplantation. After conditioning with fludarabine and melphalan, ASCT was performed. Patients were monitored for response, adverse effects, and hematopoietic reconstitution. All 7 patients completed the mobilization and leukapheresis procedures successfully and subsequently underwent conditioning and transplantation. Engraftment occurred in all patients. No major nonhematologic toxicity or transplantation-related mortality was observed. There was a significant reduction in the aberrant T cells in duodenal biopsies associated with improvement in clinical well-being and normalization of hematologic and biochemical markers (mean follow-up, 15.5 months; range, 7-30 months). One patient died 8 months after transplantation from progressive neuroceliac disease. These preliminary results showed that high-dose chemotherapy followed by ASCT seems feasible and safe and might result in long-term improvement of patients with RCD type II whose condition did not respond promptly to available drugs. (Blood. 2007;109:2243-2249)**



## Introduction

Autologous hematopoietic stem cell transplantation (ASCT) is an increasingly accepted effective treatment option for patients with severe autoimmune diseases refractory to conventional treatment[1] and has been used successfully in patients with multiple sclerosis,[2] rheumatoid arthritis,[3] systemic sclerosis,[4] systemic lupus erythematosus,[5] and Crohn disease.[6] The rationale for this strategy is based on the concept of immunoablation by intense immunosuppression using high-dose chemotherapy, with subsequent regeneration of naïve T lymphocytes derived from reinfused hematopoietic progenitor cells.[7]

In celiac disease (CD), HLA-DQ molecules bind and present gluten peptides to antigen-specific T cells. These HLA-DQ–peptide complexes induce inflammatory responses in the small intestine consisting of lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte population, hyperplasia of the crypts, and atrophy of the villi.[8] In a small percentage (2%-5%) of adult patients with CD diagnosed as adults, a refractory state develops despite strict adherence to a gluten-free diet (GFD).[9] In refractory celiac disease (RCD) the number of intraepithelial lymphocytes (IELs) is markedly raised and it is from these IELs that enteropathy associated T-cell lymphoma (EATL) may arise.[9,10] Immunophenotyping of the IELs identifies 2 groups of RCD patients: those with normal IELs (RCD I) and those with aberrant IELs, lacking surface expression of CD3 and CD8 (RCD II).[10,11] RCD II can be regarded as a "cryptic" lymphoma.[9] Strong molecular and immunophenotypic evidence now shows that a monoclonal neoplastic T-cell population may emerge from IELs in RCD. Clonal expansion of this monoclonal T-cell population eventually leads to frank EATL. The genesis and expansion of these monoclonal T cells involve both inappropriate immune responses to gluten and acquisition of genetic abnormalities. Although the monoclonal IELs in patients with RCD are neoplastic, they are not cytologically abnormal and do not form tumor masses, which differentiate these patients from those with EATL, in addition to the absence of radiologic and bone marrow evidence of lymphoma.[10,12-14]

RCD II is usually resistant to any known therapy, including azathioprine/prednisone, cyclosporine, and IL-10 therapy[15-18] and has a high risk of developing EATL (60%-80% within 5 years).[10,19] This specific type of peripheral T-cell lymphomas has a very poor outcome with 1- and 5-year survival rates in the range of 31% to 39% and 11% to 20%, respectively.[19-21] In a prospective multicenter study of 35 patients with EATL treated with 6 cycles of cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), the cumulative 2-year survival was only 28%.[11] Therefore, new treatment strategies for patients with "premalignant" CD (RCD II) are urgently needed to improve their clinical condition with the ultimate goal of resetting the immune response, which might prevent or delay development of overt EATL.

This study reports on the feasibility, safety, and efficacy of high-dose chemotherapy followed by ASCT in patients with RCD II.

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## Patients, materials, and methods

### Patients

Between March 2004 and March 2006, 13 patients were evaluated for ASCT. The 4 men and 3 women (mean age, 61.5 years; range, 51-69 years) with RCD II underwent ASCT. Six other patients were excluded because of the presence of coexistent coronary artery disease and heart failure (New York Heart Association classification III in 2 patients), EATL found on evaluation before transplantation (3 patients), and low performance status (1 patient). One patient could not be treated due to unsuccessful leukapheresis; she developed EATL and died subsequently despite chemotherapy and immunotherapy with anti-CD52 (alemtuzumab).[22] The 2 patients with congestive heart failure died from progressive disease and cachexia (first patient) and bronchiectasis (second patient). The 3 patients with EATL all died within few months, whereas the patient with low performance status died from cachexia.

The baseline characteristics of the patients are shown in Table 1. All patients received therapy with prednisone and cladribine (2-CDA) several months before undergoing ASCT (not within 6 months of transplantation). The first 3 patients (patients A, B, and C) were diagnosed with CD at relatively advanced age, had persistent diarrhea and weight loss and failed to respond to GFD, steroids, and immunosuppressives. Because of the presence of active disease and high percentage of aberrant T cells in the small bowel mucosa, they were included in this study protocol. At the age of 48 years, patient D was diagnosed with CD in association with dermatitis herpetiformis. Furthermore, he had a clinical picture of neuroceliac disease with ataxia. After exclusion of structural brain and infectious disorders, he underwent ASCT at the age of 63.5 years. Patient E has, in addition to CD with ulcerative jejunitis, Hashimoto thyroiditis, and patient F has CD with ulcerative jejunitis. One patient (patient G) was included because of the presence of very extensive ulcerative jejunitis with multiple small bowel strictures necessitating repeated resections although initially biopsies showed a low percentage of aberrant T cells. He had clinically short bowel syndrome (remaining small bowel approximately 100-150 cm) requiring total parenteral nutrition (TPN).

### Criteria for diagnosis of RCD

Patients with CD were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a GFD for at least 1 year. Furthermore, possible underlying diseases such as autoimmune enteritis, bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple disease, hypogammaglobulinemia, eosinophilic enteritis, EATL, and inflammatory bowel disease were excluded.[11] The diagnosis of RCD was established as type II when 20% or more aberrant T cells were present.[10,11,15]

### Inclusion criteria

Patients were included only when the diagnosis of true RCD with aberrant T cells was confirmed (except for patient G who was included based on the extensive ulcerative jejunitis with short bowel syndrome despite having only 10% aberrant T cells), after verifying their strict adherence to a GFD. Performance status according to the World Health Organization (WHO) criteria had to be 0 to 2, and no severe concomitant cardiac, pulmonary, renal or hepatic disease could be present. EATL was excluded by endoscopic examination with multiple biopsies, computed tomography (CT) scan, positron emission tomography (PET), and a trephine bone marrow biopsy. Furthermore, neither active uncontrolled infection nor HIV positivity was permitted.

### Evaluation

Before proceeding to ASCT, the patients were extensively evaluated as to their performance status, the presence of concomitant diseases, and extraintestinal disease or EATL. This evaluation included clinical assessment noting particularly signs and symptoms of malabsorption, body mass index (BMI), and performance according to the WHO score[23]; evaluation of adherence to a GFD including frequent consultation with dietitian (advice and follow-up) in addition to checking serology (antiendomysial [EMA] and anti–tissue transglutaminase antibody [anti-tTG], both of which usually revert to negative after strict adherence to the GFD); and evaluation by upper gastrointestinal endoscopy (UGIE), video capsule endoscopy (VCE), and double balloon enteroscopy (DBE). Duodenal biopsies (4 biopsies) were classified according to the modified Marsh criteria.[24,25] T-cell receptor (TCR) gene rearrangement study,[12-14] T-cell flow cytometry, and IEL phenotyping were performed.[15,26,27] Laboratory evaluation included whole blood cell counts and serum levels of creatinine, bilirubin, liver enzymes, lactate dehydrogenase, albumin, electrolytes, iron, ferritin, folic acid, and vitamin $B_{12}$ were determined. EMA and anti-tTG assays, HLA-DQ typing, thyroid function tests, stool examination for *Giardia* and other parasites, and HIV serology were also performed.[28] For radiologic evaluation, the patients underwent whole-body CT scanning and whole-body PET to exclude intestinal and extraintestinal localization of EATL.[29,30]

### Immunophenotyping of IELs

IELs were isolated from 3 duodenal biopsies by passing them through nylon filters ($1 \times 100$ μm, $1 \times 40$ μm, BD Biosciences, Discovery Labware, Bedford, MA). Cells were stained with fluorescent-labeled monoclonal antibodies to CD3, CD7, CD8, CD45, CD103, and TCRγδ, as well as with relevant isotype controls.

All monoclonal antibodies were from BD (BD Biosciences, San Jose, CA), except for CD103, which was from IQ Products (Groningen, The Netherlands) and analyzed by 4-color flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA). Leukocyte common antigen (CD45) was always included to identify the lymphocyte population. In some tubes cell surface CD3 staining (anti–CD3-APC) was followed by permeabilization (Cytofix/Cytoperm, BD Biosciences PharMingen, San Diego, CA) and subsequent cytoplasmic staining with anti–CD3-FITC or isotype control. Aberrant T cells were defined either as $CD7^+$ surface $CD3^-$ cells (expressed as percent of $CD103^+$ lymphocytes) or as cytoplasmic $CD3^+$, surface $CD3^-$ cells (expressed as percent of $CD103^+$ lymphocytes).[12,26]

All flow cytometry analyses were performed by an analyst and interpreted by the same medical immunologist; histopathology was performed by the same pathologist to ensure uniformity, reproducibility, and consistency of results.

### Assessment of TCR gene rearrangement by PCR

TCRγ gene rearrangements studies were performed in separate 3 to 4 duodenal specimens that were preserved on Histocon (Polysciences Europe, Eppelheim, Germany) and frozen at −20°C. DNA was extracted from cryosections of duodenal specimens by a standard procedure using proteinase-K digestion and ethanol precipitation of the gDNA. TCR-γ gene rearrangements were analyzed by multiplex polymerase chain reaction (PCR) amplification under standardized conditions. A monoclonal and polyclonal control was included in each experiment. Clonality assessment for TCR-γ gene rearrangements was done according to the Biomed-2 concerted action BM H4-CT98-3936 on PCR-based clonality studies for early diagnosis of lymphoproliferative disorders.[12-14]

### Peripheral blood stem cells mobilization and collection

Mobilization of hematopoietic progenitor cells from the bone marrow into the peripheral blood was achieved using granulocyte colony-stimulating factor (G-CSF) $2 \times 5$ μg/kg by subcutaneous injection for at least 4 days. Hematopoietic stem cells were harvested from the peripheral blood by leukapheresis and kept frozen until ASCT. The target $CD34^+$ count was more than $2 \times 10^6$/kg.

### Conditioning and ASCT

The conditioning regimen consisted of fludarabine given orally for 5 days (40 mg/m$^2$/d) and melphalan (given intravenously, 2 days, 70 mg/m$^2$/d) as shown in Figure 1. At day 0, the frozen stem cell suspension was thawed and reinfused. The rationale for this conditioning regimen was based on T-cell depletion by a purine analog combined with a modified dose of melphalan (total dose 140 mg/m$^2$) for myeloablation.

**Table 1. Baseline characteristics of the patients**

| Characteristic | Patient A | Patient B | Patient C | Patient D | Patient E | Patient F | Patient G |
|---|---|---|---|---|---|---|---|
| Age, y/sex | 62/M | 70/M | 65/F | 63/M | 64/F | 59/F | 51/M |
| Age at CD diagnosis, y | 56 | 62 | 61 | 48 | 44 | 47 | 50 |
| Age at RCD II diagnosis, y | 59 | 64 | 63 | 63 | 56 | 58 | 51 |
| Age at ASCT, y | 60 | 68 | 64 | 63 | 64 | 59 | 51 |
| Date of ASCT | Mar 2004 | Aug 2004 | May 2005 | Aug 2005 | Nov 2005 | Dec 2005 | Mar 2006 |
| HLA-DQ2 | Homozygous | Homozygous | Heterozygous | Homozygous | Heterozygous | Heterozygous | Homozygous |
| Marsh at RCD diagnosis | III-A | III-B | III-A | III-A | III-C | III-C | III-A |
| BMI, kg/m$^2$ | 19.4 | 18.9 | 17.1 | 24.1 | 20.1 | 21.3 | 20.5 |
| Performance | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| Symptoms/associations | Diarrhea, pain, weight loss | Pain, diarrhea | Diarrhea, weight loss, hypocalcemia | Diarrhea, weight loss, dermatitis, herpetiformis, neurologic symptoms (ataxia) | Weight loss, skin rash, Hashimoto thyroiditis | Weight loss, diarrhea | Diarrhea, hypocalcemia, weight loss, extensive small bowel resection |
| Serology at CD diagnosis | EMA$^+$, anti-tTG$^+$ | EMA$^+$, anti-tTG$^+$ | EMA$^+$, anti-tTG$^+$ | EMA$^+$, anti-tTG$^+$ | EMA$^+$, anti-tTG$^+$ | EMA$^+$, anti-tTG$^+$ | EMA$^+$, anti-tTG$^+$ |
| Serology at RCD diagnosis | EMA$^-$, anti-TTG$^-$ | EMA$^-$, anti-tTG$^-$ | EMA$^-$, anti-tTG$^-$ | EMA$^-$, anti-tTG$^-$ | EMA$^-$, anti-tTG$^-$ | EMA$^-$, anti-tTG$^-$ | EMA$^-$, anti-tTG$^-$ |
| Endoscopy* | Nodular mucosa | Mosaic mucosa, erosions, and ulcerations | Mosaic mucosa, visible vessels, no ulcerations | Nodular mucosa, disappearance of folds, erosions | Ulcerative jejunitis | Ulcerative jejunitis | Ulcerative jejunitis with multiple stenoses |
| CT scan | Splenic atrophy, thickened SI wall | Thickened SI loops | Splenic atrophy, dilated SI loops | Splenic atrophy | No abnormality | No abnormality | SI ileus |
| PET scan | Increased uptake in SI | Increased uptake in SI | Increased uptake in SI | No abnormality | No abnormality | No abnormality | No abnormality |

All patients were treated with prednisone and 2-CDA (cladribine).
SI indicates small intestine; tTG, tissue transglutaminase; and EMA, endomysial antibody.
*Gastroduodenoscopy (GDS), video capsule enteroscopy (VCE), or double balloon enteroscopy (DBE).

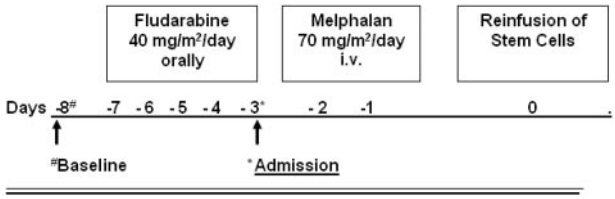


**Figure 1. Scheme of transplantation protocol.**

### Supportive care

Patients A, C, and D were supported with parenteral feeding during the 2-week period of oral mucositis after ASCT; patient G was receiving parenteral nutritional support before receiving the transplant. After discharge, all patients except patient G were able to be fed enterally. Patient G was supported to gain weight for several months with a duodenal feeding tube and limited TPN (twice a week). During admission, all patients received standard antibacterial and antifungal prophylaxis. *Pneumocystis jiroveci* pneumonia prophylaxis was initiated (trimethoprim-sulfamethoxazole gluten-free syrup 480-960 mg daily) until 6 months after transplantation. No patient received antidiarrheal or narcotic medications in the peritransplantation period. Blood and platelet transfusions were given as indicated.

### Follow-up and criteria of response

During follow-up, WHO performance status, nutritional status, and changes in weight and stool frequency were noted, as well as relevant biochemical markers. An endoscopic and histologic examination of the small intestine was performed (3, 12, and 24 months after ASCT). From the second part of the duodenum, 4 biopsies were taken for histologic assessment and 4 to 6 specimens for T-cell flow cytometry study. Hematologic data (hemoglobin, white blood cell [WBC] count, differential, and platelets) were registered before inclusion, after preconditioning, and after transplantation until recovery. The nadir WBC count, duration of neutropenia, infectious complications, bleeding tendency, and need for supportive therapies such as blood and platelet transfusions were documented.

### Ethics approval and informed consent

Approval of the medical ethics committee was obtained, and all treated patients signed an informed consent in accordance with the Declaration of Helsinki.

## Results

Table 1 summarizes the demographic and clinical characteristics of the patients before ASCT. The mean age at diagnosis of CD was 52.5 years (range, 47-62 years) and for RCD II 59 years (range, 51-64 years). Four patients were DQ2 homozygous and 3 were heterozygous.[31] The mean follow-up was 15.5 months (range, 7-30 months). All patients had a WHO performance status of 1 except patient G, whose performance status was 2. Patients B, E, F, and G had ulcerative jejunitis. Patients A, C, and D had splenic atrophy on CT scan. PET scan showed an increased uptake in the small intestine in patients A, B, and C. At the time of diagnosis of CD, all patients were positive for anti-tTG and EMA, but all reverted to negative after GFD. Before and after ASCT all patients remained negative for anti-tTG and EMA. There was no transplantation-related mortality. The conditioning regimen seems feasible in this group of patients. The mean duration of hospitalization was 19.5 days (range, 18-22 days). ASCT-related toxicity was relatively mild. Patient B had transient diarrhea and fever of undetermined origin, which was treated with intravenous antibiotics. Three weeks after discharge from the hospital, he suffered from a transient visual disturbance caused by minor retinal bleeding, which was not related to thrombocytopenia. Patient D experienced fever of undetermined origin and recovered after administration of intravenous antibiotics. One month after ASCT, patient E developed self-limiting erythematous plaque skin lesions with central necrosis. Detailed histopathologic tests excluded EATL and showed aberrant T-lymphocyte infiltration ($CD8^-CD7^+CD30^+$).

The mean time from the day of transplantation to neutrophil recovery was 17.8 days (range, 10-21 days). Only one patient (patient B) had a transient 5-day period of severe thrombocytopenia of $5 \times 10^9$/L; all other patients had nadir platelet counts between 17 and $32 \times 10^9$/L without need for platelet transfusions.

Clinical and laboratory tests before and after ASCT are shown in Table 2. Patients A, C, and D were supported with parenteral feeding during the period of oral mucositis. No patient received antidiarrheal or long-term narcotic medications. Within 3 months after ASCT, all patients showed impressive clinical improvement with normalization of stool frequency, disappearance of abdominal pain, and improvement of biochemical markers. In addition, improvement of BMI was documented (from mean 20.2 at baseline to 24.1 after ASCT). Mean serum albumin level increased from 29 g/L to 40.7 g/L. Patient G showed a remarkable clinical improvement 3 to 4 months after ASCT and was able to be fed partly enterally with parenteral nutritional support twice a week.

Table 3 shows the endoscopic and immunologic results. All patients were monoclonal for the TCR-γ. Endoscopically there was disappearance of erosions and ulcerations in the jejunum in all patients (patients B, E, F, and G) who had ulcerative jejunitis before ASCT, and histology of the small intestine showed significant regeneration as documented by down-staging of the Marsh class (patients A, B, C, E, F, and G). Overall, the aberrant ($CD7^+CD3^-$) T-cell percentage of $CD103^+$ lymphocytes decreased from a mean of 63% (range, 11%-95%) at baseline to 38% (range, 7%-68%) 3 to 4 months after transplantation. Aberrant cytoplasmic $CD3^+$ surface $CD3^-$ T-cell percentage of $CD103^+$ lymphocytes decreased from a mean of 61% (range,

**Table 2. Clinical and laboratory tests before and at the last follow-up after ASCT**

| | Patient A | | Patient B | | Patient C | | Patient D | | Patient E | | Patient F | | Patient G | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristic | Before | After | Before | After | Before | After | Before | After | Before | After | Before | After | Before | After |
| BMI, kg/m² | 19.4 | 25.6 | 18.9 | 26.1 | 22 | 24.1 | 24 | 24.1 | 20.1 | 22 | 22.1 | 23 | 20.5 | 25.8 |
| Diarrhea | Yes | No | Yes | No | Yes | Yes | Yes | No | Yes | No | Yes | No | Yes | No |
| Performance status | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 0 |
| Albumin, g/L | 32 | 43 | 33 | 41 | 30 | 46 | 32 | 41 | 20 | 41 | 30 | 44 | 26 | 46 |
| Serum iron, μM | 18 | 26 | 11 | 18 | 14 | 10 | 14 | 15 | 17 | 13 | 7 | 17 | 13 | 18 |
| Serum calcium, mM | 2.20 | 2.36 | 2.33 | 2.45 | 2.26 | 2.26 | 2.26 | 2.32 | 2.33 | 2.41 | 2.02 | 2.35 | 2.00 | 2.29 |
| Serum folic acid, nM | 10 | 44 | 10.4 | 24 | 14 | 91 | 14 | 15 | 14 | 78 | 4.4 | 35 | 29 | 18 |
| Serum $B_{12}$, pM | 470 | 560 | 169 | 307 | 440 | 290 | 440 | 790 | 206 | 666 | 107 | 317 | 269 | 221 |

Normal ranges: albumin, 34-50 g/L; iron, 10-32 μM; calcium, 2.20-2.60 mM; folic acid, > 5.9 nM; $B_{12}$, 156-672 pM. Duration of follow-up for each patient: A, 30 mo; B, 27 mo; C, 16 mo; D, 8 mo; E, 11 mo, F, 10 mo; and G, 7 mo.

**Table 3. Histological and phenotypical flow cytometric analysis of IELs in duodenal biopsies before (1-6 mo) and after ASCT**

| | Marsh category | | | | $CD7^+CD3^-$, % of $CD103^+$ ly | | | | Cyt $CD3^+$ surf $CD3^-$, % of $CD103^+$ ly | | | | $CD8^+$, % of $CD103^+$ ly | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | After | | | | After | | | | After | | | | After | | |
| Patient | Before | 3 mo | 12 mo | 24 mo | Before | 3 mo | 12 mo | 24 mo | Before | 3 mo | 12 mo | 24 mo | Before | 3 mo | 12 mo | 24 mo |
| Patient A | III-A | III-A | III-A | I | 95 | 47 | 48 | 15 | 94 | 89 | 86 | 3 | 1 | 20 | 7 | 52 |
| Patient B | III-B | I | I | I | 51 | 7 | 4 | 8 | 51 | 2 | 6 | 4 | 28 | 68 | 62 | 67 |
| Patient C | III-C | III-A | III-A | — | 62 | 33 | 24 | — | 59 | 34 | 27 | — | 15 | 41 | 36 | — |
| Patient D | III-A | III-A | — | — | 54 | 47 | — | — | 81 | 78 | — | — | 7 | 13 | — | — |
| Patient E | III-B | I | — | — | 44 | 68 | — | — | 30 | 36 | — | — | 22 | 2 | — | — |
| Patient F | III-B | III-A | — | — | 71 | 40 | — | — | 50 | 31 | — | — | 23 | 11 | — | — |
| Patient G | III-C | III-A | — | — | 11 | 30 | — | — | 10 | 27 | — | — | 63 | 52 | — | — |
| Mean | — | — | — | — | 63 | 38 | — | — | 61 | 42 | — | — | 23 | 30 | — | — |

Mean was calculated for values at 3 mo after ASCT. Normal range for Cyt $CD3^+$ surf $CD3^-$% of lymphocytes is 10% or less. TCRγ-PCR analysis showed monoclonality in all patients.

Ly indicates lymphocytes; —, not applicable; TCRγ-PCR analysis, T-cell receptor γ–polymerase chain reaction rearrangement.

10%-94%) to 42% (range, 2%-89%). Furthermore, the mean percentage of $CD8^+$ cells increased from 23% to 30% after ASCT. This was particularly noticeable in the first 3 patients. Patient D did not show a significant increase in $CD8^+$ cells and the last 3 patients have not yet shown a significant change. Individual responses to ASCT differed from each patient as shown in Table 3. Patient B showed the most impressive response with a virtual complete disappearance of aberrant T cells. The fluorescent-activated cell sorting (FACS) data from patient B are shown in Figure 2. The trend of aberrant T cells and body weight for the first 4 patients who have a follow-up period of at least 1 year is shown in Figure 3. Follow-up of patients E, F, and G is as yet limited. Two years after transplantation, our first patient (patient A) is showing further improvement in his immunopathology status as demonstrated in further decline in the percentage of aberrant T cells to 3% and histologically improved from Marsh III-A to Marsh I and the second patient (patient B) is still showing persistent complete clinical and histologic response. Patient D had no significant change in the percentage of aberrant T cells and showed no histologic improvement and no significant improvement in $CD8^+$ percentage; he died 8 months after transplantation. After ruling out structural and infectious (bacterial and viral) causes, we assumed that progressive disease of RCD II with oligoclonal T lymphocytes infiltrating the brain was the cause of death in this particular patient. EATL could not be detected. Autopsy confirmed the presence of chronic encephalitis of the right temporal lobe with T-lymphocyte infiltration. Immunohistochemistry showed that the lymphocyte infiltrate was $CD3^+$ and the majority of cells expressed CD8 positivity. TCR gene analysis showed that the T cells were oligoclonal.

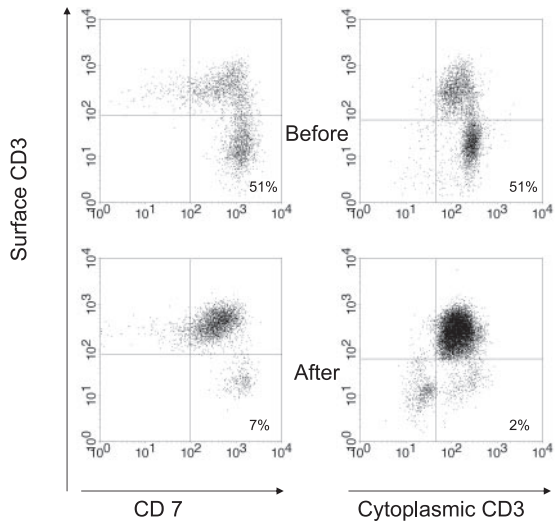


**Figure 2. Flow cytometric analysis of duodenal cells obtained from patient B, showing the change in the percentage of aberrant T-cell population before and after ASCT.** Aberrant population is shown as $CD7^+CD3^-$ within $CD103^+$ lymphocytes (left) or as cytoplasmic (cyt) $CD3^+$ surface (surf) $CD3^-$ within lymphocyte gate (right). Normal range for cyt $CD3^+$ surf $CD3^-$ % of $CD103^+$ lymphocytes is 10% or less.

## Discussion

In this pilot study, ASCT in patients with RCD II was shown to be feasible. The conditioning regimen was well tolerated in all patients and there was a substantial clinical improvement. The rapid initial response (within 3 months) and the duration (2 years in patient A and B and 14 months in patient C) of the remission up to now are promising. Complications included the occurrence of neutropenic fever in 2 patients and retinal bleeding not related to thrombocytopenia in one patient, all with full recovery. The nadir leukocyte and platelet counts and the duration of leukopenia and thrombocytopenia were comparable to our experience in patients with non-Hodgkin lymphomas and multiple myeloma undergoing ASCT after a combination of carmustine, etoposide, cytarabine, and melphalan (BEAM) or high-dose melphalan (HDM; 200 $mg/m^2$).[32] Because there is no standard conditioning regimen for ASCT used in autoimmune disease,[33] a standard regimen from our institution was used. Fludarabine induces T-cell depletion and the alkylating agent melphalan was used to achieve myeloablation.

One patient was excluded due to unsuccessful leukapheresis. Although we could achieve successful leukapheresis in all patients despite earlier 2-CDA therapy, it is possible that the reason for failure of stem cell mobilization in one particular patient might be related to the use of 2-CDA.[34] T cells play an essential role in the pathogenesis of CD and RCD II/EATL.[8,10,15] Through the activity of the enzyme tissue transglutaminase (tTG) glutamine residues in gluten are converted into glutamic acid.[35,36] Subsequently a multitude of gluten-derived peptides is generated that, when bound to either HLA-DQ2 or -DQ8 can induce T-cell responses in patients with CD.[8,24] A particular glutamine- and proline-rich 33-mer α-gliadin peptide that contains 6 different T-cell stimulatory sequences and is resistant to gastric and duodenal proteolysis might be the primary initiator of the inflammatory response to gluten. In the large majority of patients, even in children with CD, inflammatory T-cell responses to other gluten peptides are also observed, implicating multiple gluten peptides in the disease process.[26,27]

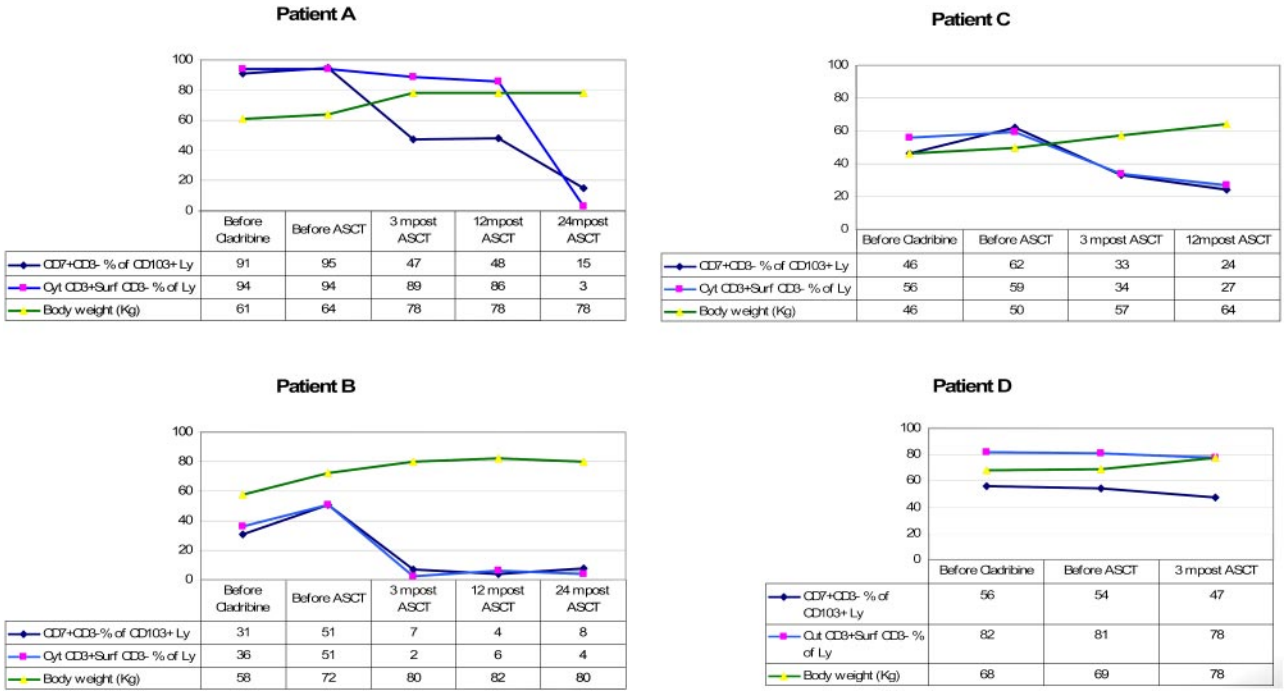


**Figure 3. The trend of aberrant T cells and body weight per patient.** Ly indicates lymphocytes; before, 1 to 3 months. Normal range for cyt $CD3^+$ surf $CD3^-$ % of lymphocytes is 10% or less.

The definition of RCD I/II has undergone refinement in recent years. It seems that the most reliable available method to differentiate between RCD I and RCD II is flow cytometry of intestinal biopsies revealing the presence of aberrant T cells. Detection of a clonal T-cell population by testing for TCR rearrangement was thought to be highly predictive of EATL development. However, oligoclonal or monoclonal IEL populations can be detected in the large majority of both RCD I and RCD II patients and also in patients who do not develop an EATL. Clonality is therefore of limited use in establishing the diagnosis of RCD and to predict the development of EATL.[14,37,38]

RCD II is usually resistant to any known immunosuppressive therapy, including azathioprine/prednisone,[15] cyclosporine,[16] and IL-10 therapy.[17] Recently, we treated 17 patients with 2-CDA on intention to induce remission. Within a mean follow-up period of 22 months (range, 7-67 months) 47% had a significant decrease in aberrant T-cell percentages with or without clinical response.[39] However, another 41% did not respond clinically, histologically, nor immunopathologically and subsequently died from EATL.

Remissions of autoimmune diseases have been described in adults after both allogenic and autologous ASCT[1-7] most probably due to the extreme immunosuppressive effects of these strategies,[1] resulting in immunoablation with subsequent regeneration of naïve T lymphocytes derived from reinfused hematopoietic progenitor cells.[7] Furthermore, recently, interesting insights into possible unsuspected mechanisms by which stem cell transplantation could affect the gut have emerged. In both animal and patient studies, sex-mismatched allogeneic stem cell transplantations have shown in both mice and women that a population of myofibroblasts derived from the donor populates the intestinal mucosa. Given the importance of myofibroblasts in orchestrating the function of epithelial cells, these data suggest a mechanism other than one targeted at immunosuppression that could beneficially reset patient functions, for example, enhancing barrier function following stem cell transplantation.[40]

These positive results, the high risk of transforming into EATL, and the absence of effective therapy for RCD with aberrant T cells led us to introduce this new strategy with the ultimate goal of resetting the immune response that might prevent or delay development of overt EATL. On follow-up, our patients showed improvement in the small intestinal histology, together with impressive clinical improvement as demonstrated by disappearance of diarrhea and abdominal pain; normalization of serum albumin, electrolytes, and hemoglobin; increase in BMI; and improvement of the performance status. Two years after transplantation, our first patient is showing further improvement in his immunopathology status as demonstrated by further decline in the percentage of aberrant T cells to 3% and histologic improvement from Marsh III-A to Marsh I. We propose that enhanced apoptosis of activated but aberrant T cells has led to this late but remarkable decline.[41] One patient died 8 months after ASCT from progressive neurologic manifestations in association with CD. Autopsy excluded any structural or infectious cause. One patient developed self-limiting erythematous plaque skin lesions with central necrosis 2 months after ASCT. Detailed analysis excluded the presence of EATL. Our most recent patient with clinically short bowel syndrome is showing remarkable clinical, endoscopic, and immunologic improvement. All our patients had negative serology before inclusion, confirming their strict adherence to GFD, and after ASCT all patients remained negative for anti-tTG and EMA. Furthermore, the first 3 patients showed a significant increase in the percentage of $CD8^+$ lymphocytes, which is seen as a marker of lymphocyte regeneration after ASCT.[42] Patient D did not show a significant increase in $CD8^+$ cells and the last 3 patients have not yet shown a significant change. Absence of a demonstrable improvement in the surface expression of CD8 on the IEL might be regarded as a poor prognostic indicator of response; this is only to be proved or disproved on longer-term follow-up.

Although the short-term results in these patients are promising, follow-up at present is too short to permit firm conclusions as to efficacy. The selection of patients for this treatment should be restricted to those patients with a substantial population of aberrant T cells, even after therapy with 2-CDA, who have a greater tendency to progress to highly lethal EATL. High-dose chemotherapy followed by ASCT seems feasible and safe and might result in long-term improvement of disease activity in RCD patients with aberrant T cells whose condition previously did not respond to

available treatments. Longer-term follow-up and additional pilot studies with larger groups of patients are needed to confirm the efficacy of this therapy.

## Authorship

Contribution: A.A.-t., O.J.V., and W.H.M.V. drafted the manuscript and provided patient care; H.M.v.R. collected and analyzed data; B.M.E.v.B. and P.E.T.S. performed T-cell flow cytometry; G.J.O., P.C.H., and C.J.J.M. revised and gave final approval of the manuscript; and C.J.J.M. supervised the management of patients before and after transplantation.

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Correspondence: Chris J. J. Mulder, VU University Medical Center, Department of Gastroenterology, PO Box 7057, 1005 MB Amsterdam, The Netherlands; e-mail: cjmulder@vumc.nl.

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ORIGINAL ARTICLE

# Auto-SCT in refractory celiac disease type II patients unresponsive to cladribine therapy

GJ Tack[1], MJ Wondergem[2], A Al-Toma[3], WHM Verbeek[1], A Schmittel[4,5], MV Machado[6], F Perri[7], GJ Ossenkoppele[2], PC Huijgens[2], MWJ Schreurs[8], CJJ Mulder[1] and OJ Visser[2]

[1]*Department of Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands;* [2]*Department of Hematology, VU University Medical Center, Amsterdam, The Netherlands;* [3]*Department of Gastroenterology and Hepatology, Sint Antonius hospital, Nieuwegein, The Netherlands;* [4]*Department of Medicine IV, Charité-University Medicine, Berlin, Germany;* [5]*Department of Hematology and Oncology, Charité-University Medicine, Berlin, Germany;* [6]*Department of Gastroenterology and Hepatology, Santa Maria hospital, Lisbon, Portugal;* [7]*Department of Gastroenterology and Digestive Endoscopy, 'Madonna del Soccorso' Hospital, San Benedetto del Tronto, Italy and* [8]*Department of Clinical Pathology, VU University Medical Center, Amsterdam, The Netherlands*

**Autologous hematopoietic SCT (auto-SCT) has been effective therapy for refractory disease, in both malignancies and severe autoimmune diseases. It seems feasible and safe for refractory celiac disease (RCD) type II, although long-term results have not been evaluated yet. With current therapies, progression into enteropathy-associated T-cell lymphoma (EATL) occurs in 60–80% patients, with a high mortality rate. Therefore, it is important to evaluate new treatment strategies. Between March 2004 and February 2010, 18 RCD II patients were evaluated for auto-SCT preceded by conditioning with fludarabine and melphalan, as a consequence of unresponsiveness to cladribine therapy. Adverse events, survival rate, EATL development and change in clinical, histological and immunological course were monitored. Thirteen patients were transplanted successfully and followed up for >2 years, 4-year survival rate was 66%. Only one patient died because of transplant-related complications. The majority of patients showed an impressive clinical improvement and five a complete histological remission. In five patients, auto-SCT could not be performed; they all died with a median survival of 5.5 months. EATL was observed in one transplanted patient, only after 4 years of follow-up. Auto-SCT after conditioning with high-dose chemotherapy in RCD II patients unresponsive to cladribine therapy is feasible and seems promising.**
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**Keywords:** auto-SCT; refractory celiac disease; enteropathy-associated T-cell lymphoma; survival; clinical course

Correspondence: Dr GJ Tack, Department of Gastroenterology and Hepatology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands.
E-mail: g.tack@vumc.nl
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## Introduction

Celiac disease (CD) is a major health care issue worldwide, with a prevalence of about 1%, affecting people of all ages.[1] A permanent state of intolerance to gluten-containing food leads to chronic inflammation of the small intestine. Hallmarks of CD are lymphocytic infiltration of the lamina propria, expansion of the intraepithelial lymphocyte (IEL) population, hyperplasia of the crypts and atrophy of the villi. CD occurs in genetically susceptible individuals who almost always carry the HLA-DQ 2/8 alleles.[2,3]

Although improvement is usually seen upon life-long withdrawal of dietary gluten, a small percentage (2–5%) of the adult-onset CD patients, especially those diagnosed above the age of 50 years, lack clinical and histological response despite strict adherence to such a gluten-free diet for more than 12 months. Patients are classified as having refractory celiac disease (RCD) if dietary consumption of gluten and other causes of malabsorption with villous atrophy are ruled out.[4,5] On the basis of immunophenotyping of the IEL, we can subdivide RCD into two groups: type I with phenotypically normal and type II with phenotypically aberrant IEL, aberrance being defined as lack of the surface markers CD3, CD4 and CD8, but with expression of cytoplasmic CD3. Clonal expansion of these aberrant IEL is thought to be responsible for progression to enteropathy-associated T-cell lymphoma (EATL).[4,6,7] The genesis and expansion of these monoclonal T cells involve both inappropriate immune responses to gluten and acquisition of genetic abnormalities. RCD II is in most patients resistant to most studied therapies, including corticosteroids, budesonide, infliximab, CYA, IL-10 and azathioprine/prednisone[8] and has a high risk of progressing to EATL (60–80% within 5 years).[9,10] This peripheral T-cell lymphoma has a very poor prognosis with a 2-year survival of only 15%, mainly because of failure to respond to chemotherapy.[9,10] Therefore, it is of utmost importance to evaluate new treatment strategies for RCD II patients, and in particular for those unresponsive to conventional

immunosuppressive drugs to improve the clinical course and prevent or delay development of EATL.

Until 2005 in our tertiary referral center for RCD, type II patients were initially treated with conventional immunosuppressive drugs, mainly azathioprine or prednisone or both, and cladribine was prescribed if they were clinically and histologically unresponsive. Cladribine is a synthetic purine nucleoside homologue with cytotoxic activity. Since 2005 a modified treatment strategy has been initiated with cladribine being drug of first choice, based on a study showing that azathioprine combined with prednisone is not effective in most RCD II patients.[11] For patients not responding, an alternative was sought. High-dose chemo/radiotherapy followed by auto-SCT has been effective therapy for refractory disease not only in hematological malignancies, but also in severe autoimmune disease.[12–14] In autoimmune disease, auto-SCT is supposed to induce immunoablation with subsequent regeneration of naive T lymphocytes derived from reinfused hematpoietic progenitor cells.[13–15] Analysis of our first seven RCD II patients treated with auto-SCT showed this treatment approach is feasible and well tolerated in celiac patients. Survival at that time seemed promising, but follow-up was short.[16]

This analysis evaluates the follow-up of 18 RCD II patients, including 7 patients of our pilot study, who were selected for auto-SCT as a consequence of unresponsiveness to conventional immunosuppressive and/or cladribine therapy. Fifteen patients were treated in Amsterdam, one each in Italy, Portugal and Germany.

## Patients and methods

This study reports extended follow-up of the open-label prospective phase I study performed by Al-Toma *et al*.[16] with six new transplanted patients added, who were referred after inclusion of this study was finished. Between March 2004 and February 2010, 18 cladribine-unresponsive RCD II patients were evaluated for auto-SCT.

### *Inclusion and exclusion criteria*

Patients aged $<70$ years and diagnosed with RCD II were included when they showed no response to one or two courses of cladribine, administered i.v. in a dose of 0.1 mg/kg per day for 5 consecutive days. Response was defined as clinical (improvement of signs and symptoms of malabsorption and weight gain) and histological (Marsh criteria 0/I) and/or immunological ($>20\%$ decrease of aberrant IEL) remission. The diagnosis of RCD II was based on persisting or recurring symptoms and small intestinal villous atrophy after a former good response despite strict adherence to a gluten-free diet for at least 1 year.[5,17] Furthermore, the clinically validated cutoff value of $>20\%$ aberrant IEL detected by flow cytometric analysis was used to distinguish RCD type I and type II.[7] A lower percentage of aberrant T cells was allowed in the presence of ulcerative jejunitis. T-cell receptor (TCR)-$\gamma$ gene rearrangement was performed as previously described.[7] Although this clonal TCR-$\gamma$ rearrangement is still a widely accepted additional method to define RCD II, Verbeek *et al*.[7] showed that the percentage of aberrant IEL detected by flow cytometric analysis is more accurate to define RCD II. EATL was excluded by several investigations after computed tomography (CT),[18] whole-body positron emission tomography,[19] magnetic resonance imaging enteroclysis,[20] upper gastrointestinal endoscopy, video capsule endoscopy[21] and/or double-balloon enteroscopy.[22] This diagnosis was confirmed according to the World Health Organization (WHO) *Classification of Tumours of Haematopoietic and Lymphoid Tissues*.[23] WHO performance status[24] had to be $\leqslant 2$, and no severe concomitant cardiac, pulmonary, renal or hepatic disease was to be present. Active uncontrolled infection and HIV positivity were also exclusion criteria.

### *PBSC mobilization, conditioning and auto-SCT*

Mobilization of hematpoietic progenitor cells from the BM into the peripheral blood was achieved using G-CSF $2 \times 10\,\mu g/kg$ by s.c. injection daily for at least 4 days without preceding chemotherapy. The minimum amount of CD34+ cells collected was $2 \times 10^6$ per kg. The conditioning regimen consisted of fludarabine administered orally for 5 days (40 mg/m$^2$ per day) and intermediate dose melphalan (administered i.v., 2 days, 70 mg/m$^2$ per day), as shown in Figure 1. At day 0 stem cells were reinfused. The purpose of this conditioning regimen was both intensive T-cell depletion and myeloablation by a purine analogue added to

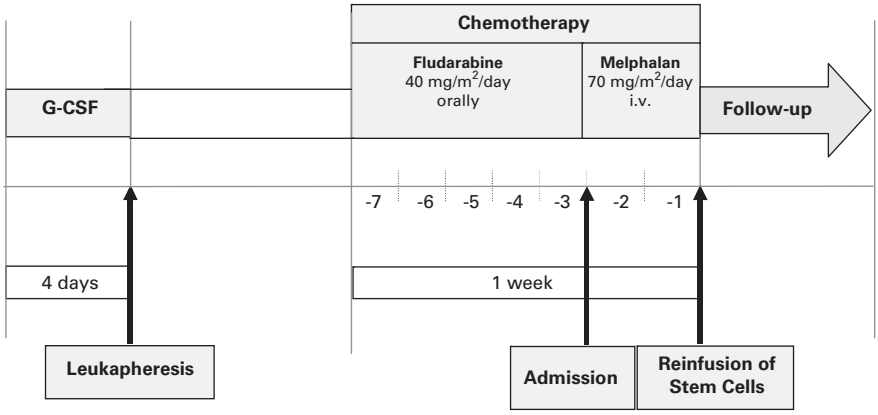


**Figure 1** Conditioning regimen before auto-SCT.

melphalan (total dose 140 mg/m$^2$). All patients received standard antibacterial and antifungal prophylaxis during neutropenia and trimethoprim–sulfamethoxazole gluten-free syrup 480–960 mg daily until 6 months after transplantation. Total parenteral nutrition and blood and platelet transfusions were given if indicated.

*Follow-up and criteria of response*

Before, during and after auto-SCT, a clinical assessment was carried out noting in particular signs and symptoms of malabsorption. Body mass index (BMI), WHO performance status, and the need for transfusions, for additional nutritional support and for additional antimicrobial treatment, were documented. Clinical remission is defined as improvement of diarrhea and constant or improved WHO performance status, combined with at least two of the following clinical parameters within the normal range or an improvement of $\geqslant 1$ point: (1) BMI; (2) albumin and (3) Hb. Multiple duodenal biopsies were taken by upper gastrointestinal endoscopy to detect histopathological abnormalities and to perform IEL immunophenotyping by flow cytometry analysis[7,25,26] at different time points (3, 12 and 24 months) after auto-SCT. Isolation of small intestinal IEL and staining for immunophenotyping were performed as previously described.[7] Complete histological remission is defined as a normalization of the architecture of the small intestinal mucosa, classified as Marsh 0 or I lesion according to the modified Marsh criteria.[27] A decline of $>20\%$ in the percentage of aberrant IEL was considered a significant immunological remission. In addition, OS and the EATL occurrence were evaluated during follow-up.

*Statistical analysis*

Quantitative data were expressed as medians. Kaplan–Meier survival curves were constructed using SPSS software (SPSS Inc., Chicago, IL, USA).

*Ethics approval and informed consent*

Approval of the medical ethics committee was obtained, and all treated patients signed an informed consent in accordance with the Declaration of Helsinki Principles.

## Results

In total, 18 RCD II patients (Table 1) who were refractory to cladribine therapy were eligible for auto-SCT. The median time between cladribine treatment and auto-SCT was 6.25 months. All patients entered the treatment protocol, however five patients did not make it to auto-SCT (Table 2 ): two due to unsuccessful leukapheresis and in three patients progression into EATL occurred before stem cells could be collected, as depicted in Figure 2. The patients reaching actual transplant ($n=13$) had a median follow-up time of $>2$ years, ranging from 10 to 67 months.

*Survival and disease status*

Out of the 18 patients unresponsive to cladribine, 8 patients died (44%), 5 of them due to EATL (28%). If auto-SCT was not reached for any reason, mortality in the nontransplanted cohort was high; all patients died within a median follow-up of 5.5 months (range 1–12.5 months). In the transplanted group, however, 23% (3 of 13) patients died. There was one transplant-related death due to septicemia with subsequent meningitis. The other two patients died of chronic encephalitis and EATL, complications associated with CD rather than transplantation. Interestingly, in the latter patient EATL occurred 4 years after transplantation (Figure 2). All the five patients in whom EATL occurred died, with a mean survival of 2 months (range 0–8 months) after diagnosis. The overall 3- and 4-year survival after undergoing auto-SCT in case of unresponsiveness to cladribine therapy was 80 and 66%, respectively (Figure 3).

Overall clinical, histological and immunological outcome is depicted in Table 3. All transplanted patients reached follow-up of almost 1 year to assess remission status. Within 1 year after auto-SCT, the majority of patients (11 of 13) showed impressive clinical improvement with normalization of stool frequency, disappearance of gastrointestinal symptoms and normal levels of or improvement of $\geqslant 1$ point in BMI, albumin and/or Hb. All patients had a WHO performance status of 0 at the end of follow-up. In addition, improvement of BMI was documented from a median level of 20.1 kg/m$^2$ at baseline to 22.5 kg/m$^2$ after auto-SCT. The median serum albumin level increased from 36 to 42 g/L (Table 3).

**Table 1** Patient characteristics

| | *Transplanted* (n = *13*) | *Nottransplanted* (n = *5*) |
|---|---|---|
| *Gender* | | |
| Female/Male | 7:6 | 3:2 |
| *Age at CD diagnosis (years)* | | |
| Median (range) | 50 (37–68) | 63 (45–66) |
| *Age at RCDII diagnosis (years)* | | |
| Median (range) | 58 (42–68) | 64 (47–70) |
| *Age at (intention to) auto-SCT (years)* | | |
| Median (range) | 59 (43–68) | 65 (52–70) |
| *Treatment before auto-SCT* | | |
| Cladribine | 13 | 5 |
| Azathioprine/Prednisone | 3 | 2 |
| *Time between cladribine and auto-SCT (months)*[a] | | |
| Median (range) | 6.25 (3–30) | |
| *Follow-up time (months)* | | |
| Median (range) | 26 (10–67) | 5.5 (1–12.5) |
| *HLA-DQ status* | | |
| DQ2 heterozygous | 4 | 2 |
| DQ2 homozygous | 8 | 2 |
| DQ2+ and DQ8+ | 1 | 1 |
| *TCR-γ rearrangement* | | |
| Monoclonal | 8 | 4 |
| Oligoclonal | 5 | 1 |

Abbreviation: CD = celiac disease.
[a]Due to change in treatment strategy two patients were transplanted 2 years after cladribine therapy.

**Table 2** Transplant characteristics

| *Patient* | *Duration admission (days)* | *Reinfusion CD34+ cells ($\times 10^6$ per kg)* | *Time to neutrophil recovery (days)*[a] | *Time to platelet recovery (days)*[b] | *TPN* | *Platelet transfusion* | *RBC transfusion* |
|---|---|---|---|---|---|---|---|
| 1 | 19 | 2.09 | 12 | 11 | Yes | 1 × | 3 × |
| 2 | 17 | 2.21 | 12 | 11 | Yes | 2 × | 6 × |
| 3 | 14 | 2.06 | 12 | 12 | Yes | 1 × | No |
| 4 | 24 | 2.0 | 17 | 27 | No | No | No |
| 5 | 16 | 2.26 | 7 | 7 | No | 1 × | 6 × |
| 6 | 29 | 2.21 | 18 | 17 | Yes | No | 3 × |
| 7 | 23 | 2.47 | 19 | 17 | Yes | No | 1 × |
| 8 | 24 | 2.17 | 13 | 14 | No | 1 × | 6 × |
| 9 | 19 | 2.96 | 16 | 15 | No | No | No |
| 10 | 25 | 2.4 | 20 | 22 | No | 1 × | 2 × |
| 11 | 30 | 3.6 | 13 | 8 | Yes | No | 2 × |
| 12 | 28 | 3.26 | 12 | 9 | Yes | No | No |
| 13 | 19 | 2.27 | 14 | 12 | No | 1 × | No |
| Median (range) | 23 (14–30) | 2.26 (2–3.6) | 13 (7–20) | 12 (7–27) | | | |

Abbreviation: TPN = total parenteral nutrition.
[a]Neutrophil recovery: time to achieve a neutrophil count above $0.5 \times 10^9$/L.
[b]Platelet recovery: time to platelet count above $20 \times 10^9$.

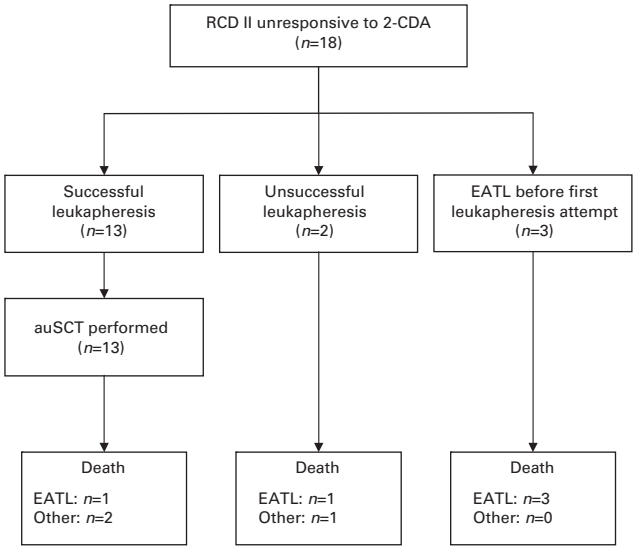


**Figure 2** Flow chart of cladribine-resistant RCD II patients.

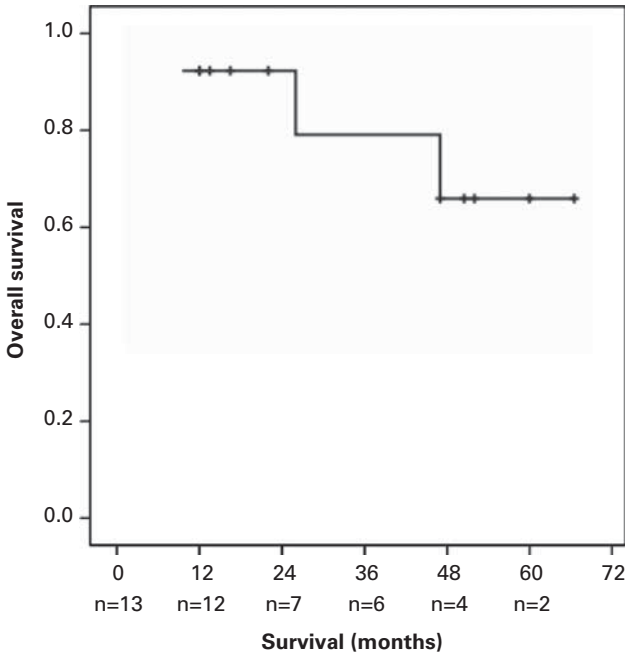


**Figure 3** Survival curve of RCD II patients unresponsive to cladribine therapy who were treated with high dose of chemotherapy followed by auto-SCT.

In total, 38% (5 of 13) had a complete histological remission, defined as Marsh 0 or I. Two of them achieved this remission within 3 months, two within 6 months and one after 2 years. One patient had a PR from a Marsh IIIB to Marsh II lesion and two patients already had a Marsh 0 and I lesion before auto-SCT. The remaining two patients showed stable and progressive histological abnormalities after auto-SCT. The median percentage of immunophenotypically aberrant intestinal T lymphocytes was 45% before transplantation and 54% at the end of follow-up. Only one patient showed a decrease in the percentage of aberrant T lymphocytes to within the normal value. The patient showed a clinical and complete histological remission as well.

## Discussion

Till now there is no standardized treatment strategy for RCD II.[8] The high risk of subsequent progression to EATL with dismal prognosis highlights the need for new treatment strategies. Although treatment with conventional immunosuppressive drugs, including azathioprine and prednisone, may lead to clinical remission in several RCD II patients,[11,28,29] most of them do not have complete histological improvement, do not have a beneficial survival due to progression into EATL[11,30] and/or become steroid dependent.[28] Therefore, in our medical center cladribine rather than azathioprine is prescribed, showing similar clinical improvement, but with better histological response rates without any steroid dependency and need for long-term medication.[31] However, approximately half of the patients is still clinically and histologically unresponsive to this

**Table 3** Overview of the clinical, histological and immunological results before and at the end of follow-up

| *Patient* | *Alive* | *Clonality* | *WHO performance status* | | *BMI (kg/m²)* | | | *Albumin (g/L)* | | | *HB (mmol/L)* | | | *Marsh score* | | | *Aberrant IEL (%)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *before* | *after* | *before* | *<1 year* | *>1 year*[a] | *before* | *<1 year* | *>1 year* | *before* | *<1 year* | *>1 year* | *before* | *<1 year* | *>1 year* | *before* | *<1 year* | *>1 year* |
| 1 | Yes | Oligoclonal | 0 | 0 | 20 | 25.4 | 26.7 | 34 | 43 | 40 | 7.5 | 7.6 | 7.4 | IIIA | 0 | 0 | 41 | 62 | 65 |
| 2 | No[b] | Monoclonal | 0 | 0 | 19.9 | 24.5 | 25.5 | 37 | 40 | 25 | 8.0 | 8.8 | 7.4 | IIIB | IIIA | I | 92 | 86 | 37 |
| 3 | Yes | Monoclonal | 0 | 0 | 20.8 | 22.5 | 22.5 | 28 | 34 | 42 | 6.3 | 7.7 | 8.6 | I | 0 | 0 | 92 | 91 | 91 |
| 4 | Yes | Oligoclonal | 0 | 0 | 24.5 | 25.2 | 29.1 | 36 | 29 | 40 | 7.1 | 8.9 | 9.6 | II | 0 | 0 | 45 | 6 | 6 |
| 5 | Yes | Oligoclonal | 1 | 0 | 20.1 | 20.1 | 20.4 | 39 | 42 | 42 | 6.6 | 7.1 | 7.2 | II | 0 | 0 | 30 | 28 | 40 |
| 6 | Yes | Monoclonal | 0 | 0 | 19.1 | 22.7 | 22.9 | 29 | 46 | 48 | 7.5 | 7.8 | 7.2 | IIIB | IIIA | II | 55 | 47 | 65 |
| 7 | No[c] | Monoclonal | 1 | 0 | 21.2 | 19.1 | 20.0 | 41 | 41 | 43 | 6.2 | 7.4 | 5.2 | 0 | IIIA | I | 1[b] | 4 | ND |
| 8 | Yes | Monoclonal | 1 | 0 | 21.2 | 22.3 | 22.7 | 27 | 41 | 43 | 6.4 | 7.4 | 7.1 | 0 | I | I | 20 | 48 | 53 |
| 9 | No | Monoclonal | 0 | 0 | 23.5 | 23.5 | — | 32 | 44 | — | 7.9 | 8.4 | — | IIIB | IIIA | — | 81 | 78 | — |
| 10 | Yes | Oligoclonal | 1 | 0 | 19.2 | 18.4 | 18.4 | 36 | 41 | 44 | 8.9 | 7.6 | 8.9 | IIIB | IIIA | IIIA | 86 | ND | ND |
| 11 | Yes | Monoclonal | 2 | 0 | 17.9 | 16.8 | — | 36 | 36 | — | 5.2 | 6.9 | — | IIIC | 0 | — | 60 | 78 | — |
| 12 | Yes | Oligoclonal | 1 | 0 | 18.8 | 20.3 | — | 26 | 40 | — | 6.9 | 8.2 | — | 0 | 0 | — | 40 | 48 | — |
| 13 | Yes | Monoclonal | 0 | 0 | 20.1 | 20.1 | — | 40 | 47 | — | 8.6 | 8.7 | — | 0 | I | — | 40 | 56 | — |

Abbreviations: BMI = body mass index; ND = not determined; WHO = World Health Organization.
[a]Results at the end of follow-up, but after at least 1 year.
[b]In this patient progression into EATL occurred.
[c]This patient had persisting ulcerative jejunitis, which is characterized by a low percentage of aberrant IEL detected by flow cytometry.

therapy. In our analysis this specific group of RCD II patients refractory to cladribine therapy was scheduled for treatment with high-dose chemotherapy followed by auto-SCT.

In agreement with our pilot study,[16] we report in this larger cohort of RCD II patients that the treatment approach we described is feasible and no serious transplant-related short-term adverse events were noted. Moreover, during extended follow-up no secondary malignancies and myelodysplastic disorders were observed. Treatment-related mortality was not increased compared to other indications for auto-SCT.

The 5-year survival reported for RCD II, irrespective of treatment is currently 44–58%.[9,28,29] In these series, patients were initially treated with different types of medication, mainly conventional immunosuppressive drugs. In our analysis however, only RCD II patients unresponsive to these treatment options were scheduled for auto-SCT, having a 4-year survival of 66% if they manage to proceed transplantation. This survival rate seems promising so far, as this specific group of unresponsive patients has a worse prognosis. In addition, this is supported by the high mortality of the five refractory patients who could not be transplanted.

Four patients were already diagnosed as having overt EATL before first leukapheresis attempt, reflecting the quick development of this condition. The EATL rate observed in the transplanted group (1 of 13) is much better than that reported in published RCD II series (60–80%),[32] however, in a small series. Even if we include the nontransplanted patients who developed EATL, the EATL rate is less than reported (30%). As one transplanted patient developed an overt EATL only after 4 years of follow-up, chemotherapy followed by auto-SCT might possibly delay the development of this type of lymphoma. Whether progression into EATL is prevented or delayed must be elucidated by more prolonged follow-up.

In addition, in two patients transplantation could not be performed because of failure of stem cell collection, most likely as a consequence of previous administration of cladribine therapy. Both cladribine and fludarabine are purine nucleoside analogues. Although fludarabine-containing regimens are well known for impairing stem cell mobilization, less is known about the influence of cladribine on mobilization efficacy.[33] However, it seems from our data that cladribine might indeed influence stem cell collection. A solution might be mobilization after single-dose CY together with G-CSF and/or implementing plerixafor.[34] This new approach should be evaluated in this patient group.

Our study results showed an impressive clinical improvement and enhanced quality of life in almost all patients after transplantation. Approximately half of the patients had a significant recovery of the architectural abnormalities of the small intestinal mucosa. It is intriguing that a high percentage of aberrant IEL persists after auto-SCT, particularly in view of the improved Marsh score. These aberrant T cells reside in the intraepithelial as well as the lamina propria layer.[35] Although this percentage is crucial in the diagnostic work-up for distinguishing RCD types I and II,[7] our data suggest that the percentage of aberrant

IEL is not suitable for monitoring therapy and predicting prognosis, at least in this cohort and time of follow-up. In fact, the percentage of aberrant T cells is not the same as the overall depletion of T cells after auto-SCT. Whether the absolute aberrant T-cell load, which might be quantified by flow cytometry or by quantitative positron emission tomography scanning, instead of the percentage of aberrant T cells is more suitable to predict prognosis is not clear yet. If histopathology or another undefined parameter should be used to quantify mass needs to be investigated as well. Furthermore, clonal expansion of these aberrant IEL is considered to be responsible for progression into an EATL, however in our series the persisting high percentage of aberrant IEL after auto-SCT was not reflected in increased EATL development. In addition, the TCR-$\gamma$ rearrangement performed after auto-SCT could not detect a clonal peak with current available technology (data not shown). So far, it is still generally accepted that aberrant T cells are the factor responsible in EATL development. Thus, further reduction of T cells or T-cell mass by intensifying conditioning pre-auto-SCT might improve outcome. This could be achieved by a higher dose of fludarabine/cladribine combined with anti-CD52 (alemtuzumab), anti-thymocyte globulin or new specific anti-T-cell agents.

Randomized clinical trials comparing conservative follow-up to auto-SCT, if unresponsive to cladribine therapy, are not available so far. In the future, multicenter randomized trials or trials with historical control groups will be needed to explore these new treatment strategies.

In conclusion, high-dose chemotherapy followed by auto-SCT in an RCD II patients unresponsive to cladribine therapy is well tolerated and no clinically relevant long-term complications were found so far. Moreover, if the patients manage to proceed auto-SCT, an impressive clinical and histological improvement is obtained, and survival rates so far are promising probably because of less progression to EATL.

## Conflict of interest

The authors declare no conflict of interest.

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Alimentary Tract

# Disappointing outcome of autologous stem cell transplantation for enteropathy-associated T-cell lymphoma

A. Al-toma [a], W.H.M. Verbeek [a], O.J. Visser [b], K.C. Kuijpers [c], J.J. Oudejans [d], H.C. Kluin-Nelemans [e], C.J.J. Mulder [a,*], P.C. Huijgens [b]

[a] *Department of Gastroenterology, VU University Medical Centre, Amsterdam, The Netherlands*
[b] *Department of Haematology, VU University Medical Centre, Amsterdam, The Netherlands*
[c] *Department of Clinical Pathology, VU University Medical Centre, Amsterdam, The Netherlands*
[d] *Department of Clinical Pathology, St. Antonius Hospital, Nieuwegein, The Netherlands*
[e] *Department of Haematology, Groningen Medical Centre, The Netherlands*

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**Abstract**

**Background.** Despite treatment, enteropathy-associated T-cell lymphoma has a very poor outcome. Chemotherapy can be complicated by small bowel perforation, gastrointestinal bleeding and development of enterocolic fistulae. Here we report on the feasibility, safety and efficacy of high-dose chemotherapy followed by autologous stem cell transplantation in patients with enteropathy-associated T-cell lymphoma (three upfront and one at relapse), with or without prior partial small bowel resection.

**Methods.** Four patients [two males, two females, mean age 65 years (range 60–69 years)] received high-dose chemotherapy followed by autologous stem cell transplantation. Partial small bowel resection has been performed in three patients.

**Results.** All four patients completed the mobilization and leucopheresis procedures successfully and subsequently received conditioning chemotherapy and transplantation. Engraftment occurred in all patients. No major non-haematological toxicity or transplantation-related mortality was observed. One patient has ongoing complete remission 32 months after transplantation. Three patients died from relapse within few months after autologous stem cell transplantation.

**Conclusions.** Autologous stem cell transplantation seems unsatisfactory for patients with enteropathy-associated T-cell lymphoma. More intensive conditioning and aggressive chemotherapy with/or without targeted immunotherapy as well as allogenous stem cell transplantation needs to be explored.


*Keywords:* Autologous stem cell transplantation; Coeliac disease; Enteropathy-associated T-cell lymphoma

## 1. Introduction

Enteropathy-associated T-cell lymphoma (EATL) is a specific type of peripheral T-cell lymphoma associated with coeliac disease, and it is known for its very poor outcome: 1- and 5-year survival rates in the range of 31–39% and 11–20%, respectively [1,2]. In a prospective, multicentre study of 35 patients with EATL treated with six cycles of cyclophosphamide, doxorubicine, vincristine and prednisone (CHOP), the cumulative 2-year survival was only 28% [3].

EATL is rare, except in the coeliac disease population, where the risk has been estimated to be as high as 19.2 times that of the general population [4]. The annual incidence rate of EATL has been reported to be 0.5–1 per million people in Western countries [5].

Strict adherence to a gluten-free diet for more than 5 years has been shown to reduce the overall cancer risk, particularly EATL, in the coeliac disease group to that of the general population [6].

* Corresponding author at: Department of Gastroenterology en Hepatology, VU University Medical Centre, P.O. Box 7057, 1007MB Amsterdam, The Netherlands. Tel.: +31 20 4440613; fax: +31 20 4440554.
*E-mail address:* cjmulder@vumc.nl (C.J.J. Mulder).

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EATL can present in two different clinical patterns. There are patients with well-established coeliac disease who have responded to a gluten-free diet but then deteriorate because of the development of refractory coeliac disease (RCD) or EATL. In the other group, patients are not known with coeliac disease; and the diagnosis of both coeliac disease and EATL is made more or less simultaneously (*de novo* EATL) [7].

An immunophenotypically aberrant clonal intraepithelial T-cell population has been found in up to 75% of patients with RCD [8]. Clonal T-cell receptor (TCR) gene rearrangements have been found in patients with RCD without histologic evidence of lymphoma [9–11]. It remains unclear whether chronic inflammatory conditions such as coeliac disease provoke an aberrant immune response or the underlying abnormal T-cell response is already present, creating the picture of RCD [12]. Furthermore, identifying patients at risk can be difficult, as establishing the diagnosis of RCD itself takes time [13].

In the largest case series reporting on treatment and clinical outcome in EATL, more than half of the patients could not complete treatment due to poor nutritional status and chemotherapy-induced small bowel perforations, gastrointestinal bleeding and development of enterocolic fistulae [14].

There have been few case studies of EATL patients treated with chemotherapy and upfront autologous stem cell transplantation (ASCT) [1,14–18]. These reports described very small groups of patients who, after reaching complete remission (CR), had a disease-free survival ranging from 0 to 64 months after ASCT. Encouraging results came from a recent report [18] describing the treatment of six patients with upfront ASCT; four of the patients remained alive in CR at 1.83–4.32 years; two had relapse.

Here we report on the feasibility, safety and efficacy of high-dose chemotherapy followed by ASCT in patients with EATL (three upfront and one at relapse), with or without prior partial small bowel resection.

## 2. Patients and methods

### 2.1. Patients

Four patients (two males, two females) with a diagnosis of EATL received high-dose chemotherapy followed by ASCT.

Patient characteristics are summarized in Table 1.

Patient A is a 69-year-old female, known to have mononeuritis multiplex and Sjögren syndrome for more than 20 years. At the age of 64 years, a diagnosis of EATL and coeliac disease was established. She was treated with gluten-free diet and partial small bowel resection followed by chemotherapy, which consisted of eight-course CHOP therapy (without vincristine because of the presence of peripheral neuropathy). Thereafter, she remained in CR for 18 months. Subsequently, she developed relapse with localization in the jejunum. A second resection was necessary, and after recovery, second line chemotherapy was initiated, consisting of dexamethasone, cytarabine and cisplatinum (DHAP, two cycles), and etoposide, ifosfamide and methotrexate (VIM, one cycle; DHAP–VIM–DHAP). This treatment was followed by high-dose chemotherapy consisting of BCNU, etoposide, cytarabine and melphalan (BEAM) and ASCT.

Patient B is a 60-year-old female who was diagnosed with *de novo* EATL localized in the mesenteric lymph nodes. She was treated with four-cycle CHOP chemotherapy and gluten-free diet. Subsequently, she received fludarabine

Table 1
Patients' characteristics

| | Patient A | Patient B | Patient C | Patient D |
|---|---|---|---|---|
| Age/gender | 69/female | 60/female | 66/male | 66/male |
| Age CD (years) | 64 | 60 | 65 | 65 |
| Age ASCT (years) | 66 | 60 | 66 | 65 |
| HLA-DQ 2 haplotype | Homozygous | Homozygous | Heterozygous | Heterozygous |
| Marsh at Dx EATL | IIIA | IIIB | IIIA | IIIA |
| Percent aberrant T cells at Dx | 50 | 51 | 30 | NA |
| Immunohistochemical type of EATL | $CD3^+CD8^-CD30^+$ | $CD3^+CD8^-CD30^+$ | $CD3^+CD8^-CD30^+$ | $CD3^+CD8^+CD30^-$ |
| Extraintestinal localization | Mesenteric lymph nodes | Mesenteric lymph nodes | Mesenteric lymph nodes | Hilar, retroperitoneal and mesenteric nodes |
| Bone marrow involvement | No | Yes | No | No |
| Endoscopy (GDS, VCE, DBE, coloscopy) | Ulcerative jejunitis | Diffuse ulcerative jejunitis | Scalloping of folds | Ulcerative jejunitis, ulcerations in colon |
| CT scan/MR enteroclysis | Dilated second part with abrupt narrowing of distal duodenum | Diffuse lesions in both lungs | Dilated small bowel segment with localized thickening | Mesenteric lymphadenopathy with thickened jejunum loop |
| FDG-PET scan | Increased activity in upper abdomen | Increased activity in right lung and neck | Increased activity in upper abdomen | Increased activity in left hilar region |

At diagnosis all patients have stage IV disease and positive TTG, EMA. TTG = serum anti-tissue transglutaminase; EMA = serum anti-endomysial antibodies; CD = coeliac disease; GDS = gastroduodenoscopy; VCE = video capsule endoscopy; DBE = double-balloon enteroscopy.

(40 mg/m$^2$/day for 5 days) and melphalan (70 mg/m$^2$ at day −2 and day −1) followed by ASCT.

Patient C is a 66-year-old male. He was admitted because of pain in the epigastric region and weight loss. On computed tomography (CT) scan of the abdomen, localized thickening of the small bowel wall was seen. He underwent 1 m *en bloc* resection of small bowel segment and mesentery with primary anastomosis. Histopathologically, the diagnosis of EATL was confirmed. Subsequently, he was treated with CHOP chemotherapy (eight courses in total). Because of partial response after four courses (radiologic analysis showed multiple mesenteric lymph nodes), consolidation with fludarabine and melphalan was administered, followed by ASCT.

Patient D is a 66-year-old male, known with coeliac disease and osteoporosis. He was on gluten-free diet for 1 year. Video capsule enteroscopy (VCE) and double-balloon enteroscopy (DBE) were performed because of persistent weight loss. Both these methods showed ulcerative jejunitis of the distal jejunum and ileum, but histopathology of endoscopic biopsies was not conclusive. An emergency laparotomy was performed because of persistent melena and haemodynamic instability. Partial resection of small intestine was performed. In the resection specimen, multiple localisations of a lymphoma had been identified in the wall of the ileum.

After recovery from laparotomy, he was treated with eight-cycle CHOP chemotherapy, combined with immunotherapy (anti-CD52, alemtuzumab). Subsequently, he received BEAM, followed by ASCT.

### 2.2. Staging

The Ann Arbor staging system was used based on clinical assessment, chest X-ray, whole body CT scan, positron emission tomography (FDG-PET) [19], magnetic resonance (MR) enteroclysis, evaluation by an ear nose throat surgeon, and bone marrow trephine biopsy.

### 2.3. Criteria of diagnosis

The diagnosis of EATL was established according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues [20]. The immunohistochemical features of EATL are the presence of large or medium-sized T-cell proliferation expressing a $CD3^+$ $CD8^{+/-}$ and $CD103^+$.

EATL can be $CD3^+CD8^-CD30^+$ large cell lymphomas, $CD3^+CD8^+CD30^-$ small cell lymphomas or γδ-lymphomas. Diagnosis of EATL was confirmed by an expert panel of pathologists.

### 2.4. Peripheral blood stem cells mobilization and collection

Mobilization of haematopoietic progenitor cells from the bone marrow into the peripheral blood was performed using granulocyte colony-stimulating factor (G-CSF).

Haematopoietic stem cells were collected from the peripheral blood by leucopheresis.

### 2.5. Response criteria

Response to therapy was evaluated according to the Cheson criteria [21]. These criteria include anatomic definitions of response.

*A complete response* requires the following:

1. Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities (e.g. lactate dehydrogenase) definitely assignable to NHL.
2. All lymph nodes and nodal masses must have regressed to normal size.
3. The spleen must have regressed in size and must not be palpable on physical examination.
4. Bone marrow, if positive at baseline, must be histologically negative for lymphoma.

*Complete response, unconfirmed* (*CRu*) includes those patients who fulfil criteria 1 and 3 above, but with one or more of the following features:

- A residual lymph node mass greater than 1.5 cm in greatest transverse diameter that has regressed by more than 75% in the size. Individual nodes that were previously confluent must have regressed by more than 75% in their size compared with the size of the original mass.
- Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

*A partial response* (PR) requires the following:

- More than 50% decrease in size of the six largest dominant nodes or nodal masses.
- No increase in the size of the other nodes, liver or spleen.
- Splenic and hepatic nodules must regress by at least 50% in size.
- With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.
- No new sites of disease.

*Stable disease* is defined as less than a PR (as described above) but not progressive disease (see below). *Progressive disease* is defined as 50% increase from the nadir in the size of any previously identified abnormal node or appearance of any new lesion during or at the end of therapy.

## 3. Results

### 3.1. Patient characteristics

The baseline characteristics of the four patients are shown in Table 1. The mean age was 65 years (range 60–69 years).

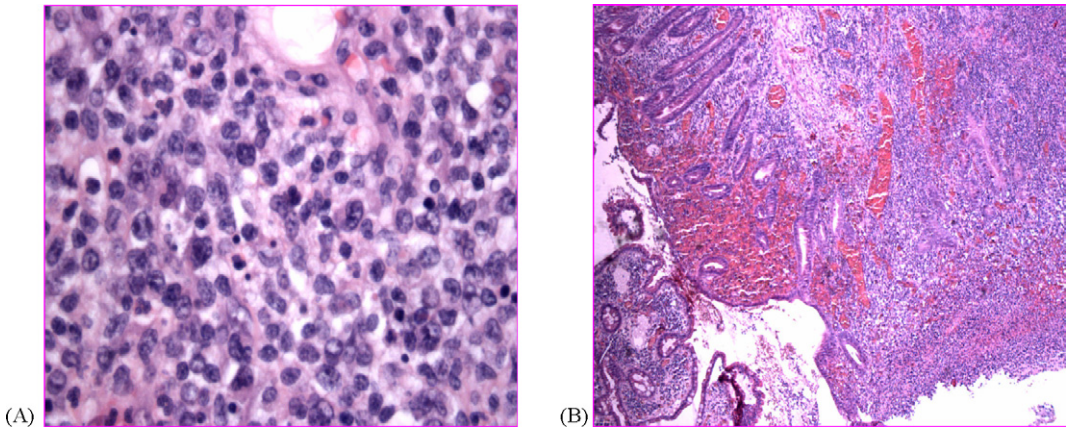

Fig. 1. EATL in ileum resection specimen with a proliferation of middle-sized atypical lymphocytes (A) leading to villous atrophy and ulceration (B) (H&E, 12.5× and 630×).

Three patients had *de novo* type of EATL, while one patient was known to have coeliac disease 1 year before developing EATL (patient D). All patients had positive serology for coeliac disease at diagnosis.

### 3.2. Endoscopical, histological and immunophenotypical features

Endoscopically, three patients had evidence of ulcerative jejunitis. All patients had definite histological features of coeliac disease according to the Marsh criteria (three had Marsh IIIA and one IIIB). A significant percentage (30–51%) of the intraepithelial lymhocytes was aberrant, defined as $CD7^+$ surface $CD3^-$ cells (expressed as percent of $CD103^+$ lymphocytes) or cytoplasmic $CD3^+$, surface CD3 negative cells (expressed as percent of $CD103^+$ lymphocytes) in all patients.

Immunophenotypical testing showed that these malignant cells were $CD3^+CD8^-CD30^+$ in three patients (patients A, B and C) and $CD3^+CD8^+CD30^-$ in 1 patient (patient D).

The histological and immunophenotypical features of patient D are shown in Figs. 1 and 2, respectively.

### 3.3. Stem cell mobilization

Mobilization of haematopoietic progenitor cells from peripheral blood was achieved successfully and uncomplicated in all patients using G-CSF.

### 3.4. Transplantation-related toxicity

There was no transplantation-related mortality or serious morbidity. The mean duration of hospitalization was 20 days (range 18–24 days). Haematopoietic recovery was fast in all patients. The median time to reach neutrophils $>0.5 \times 10^6$/l and unsupported platelets $>20 \times 10^6$/l were 12 days (8–15) and 14 days (9–16), respectively.

### 3.5. Response and survival after ASCT

Table 2 summarizes the responses to treatments. One patient (patient A) was in remission for 18 months after standard chemotherapy and received ASCT in second CR. She is in ongoing CR at 32 months.

Patient B developed severe neurological complaints at 4 weeks after ASCT before response to transplantation could be assessed. A cauda equina syndrome was diagnosed. CSF examination confirmed the presence of lymphoma cells carrying the same immunological markers ($CD3^+CD8^-CD30^+$). She received palliative radiotherapy. Unfortunately, she died from this rapidly progressing CNS relapse 2 months after ASCT.

Patient C was admitted 6 months after ASCT because of persistent gastrointestinal bleeding and pancytopenia. He has partial remission after chemotherapy and subsequently CR after ASCT. He received supportive care (blood and platelet transfusions). Relapsed EATL was suspected, but all supportive care was withdrawn after explicit request of the patient and his family, and he died several days later. Autopsy examination showed the presence of a large amount of blood in the gastrointestinal tract and multiple mesenteric pathological lymph nodes (Fig. 3). Microscopic examination of these nodes confirmed the presence of EATL in relapse.

Patient D had shown initial CR after chemotherapy and remained so after ASCT, but he developed a relapse 9 months after transplantation. He was admitted with bleeding per rectum. Colonoscopy showed multiple deep ulcerations in the ascending colon and terminal ileum. Histopathologic examination confirmed EATL relapse. He died and an autopsy examination showed the presence of a large amount of blood in the gastrointestinal tract due to numerous ulcers in the small intestine and the colon with multiple mesenteric pathological lymph nodes. Microscopic examination of these nodes showed considerable depletion of lymphocytes, but the pathological lymphoid population could not be identified with certainty in the lymph nodes.

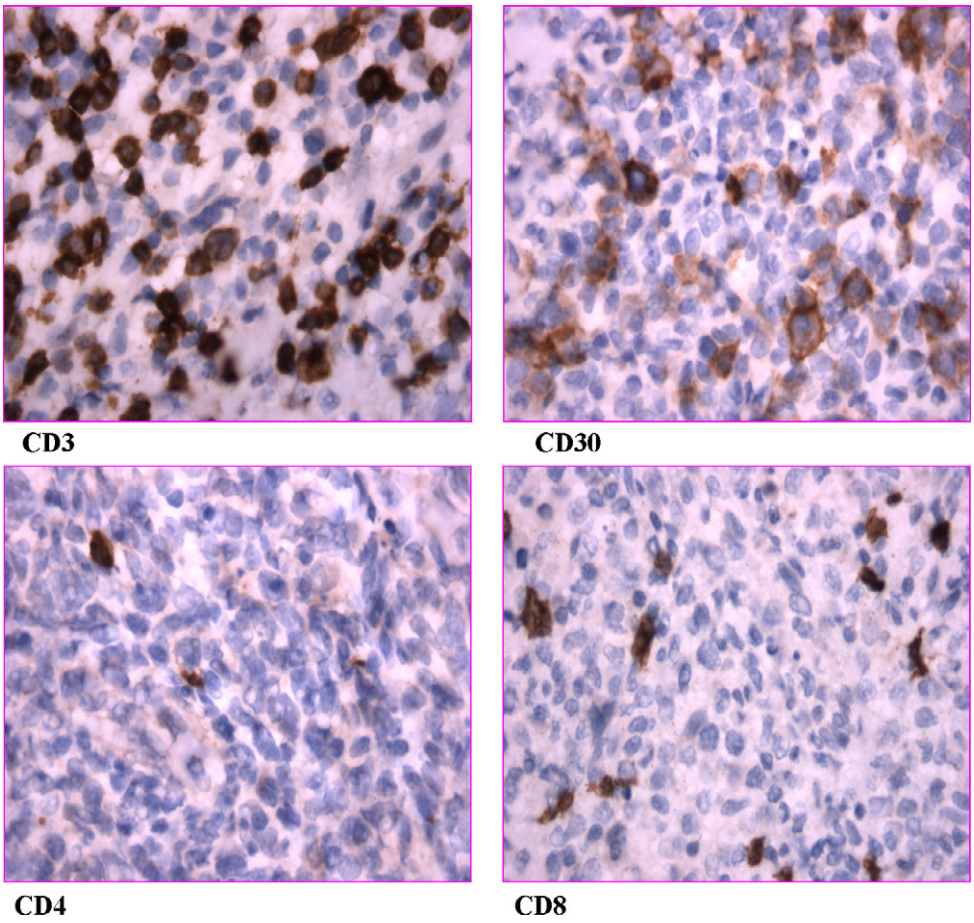


Fig. 2. EATL in ileum resection specimen expressing CD3 and partly also CD30. Residual small T lymphocytes express CD4 or CD8, lymphoma cells virtually all negative.

Table 2
Treatment results

| | Patient A | Patient B | Patient C | Patient D |
|---|---|---|---|---|
| Follow up (months) | 34 | 2 | 6 | 9 |
| Resection performed | Yes | No | Yes | Yes |
| Response to CHOP | CR (18 months) followed by relapse | CR | PR | CR |
| Other therapies | DHAP–VIM–DHAP | – | – | Alemtuzumab |
| Preconditioning | BEAM | Flu + Mel | Flu + Mel | BEAM |
| Relapse after ASCT | No | Yes | Yes | Yes |

CR = complete remission; PR = partial remission; Flu + Mel = fludarabine and melphalan.

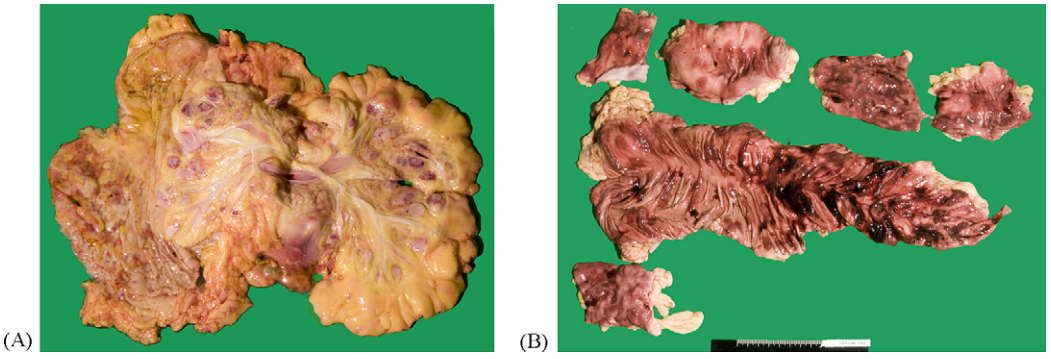


Fig. 3. Macroscopic picture showing multiple pathological mesenteric lymph nodes (A) and large amount of blood in the small intestine (B).

## 4. Discussion

Intestinal T-cell lymphomas or EATLs respond poorly to available anti-lymphoma regimens. Results are better in patients with limited stages of disease [22]. The most frequent complications of treatment are small bowel perforation, obstruction, gastrointestinal bleeding and infection.

We report here our results of the treatment of four patients with an advanced stage of EATL diagnosis (stage IV). The induction chemotherapy and "debulking" were feasible, as the patients were in good condition before undergoing ASCT. The conditioning regimen and ASCT were well tolerated in all patients. The nadir leucocytes and platelets counts and the duration of leucopenia and thrombocytopenia were comparable to that in patients with other types of non-Hodgkin lymphomas and multiple myeloma receiving ASCT after BEAM or high-dose melphalan [23].

There was no transplantation-related mortality. Our first patient received ASCT at second complete response, while the other three received upfront ASCT. These three patients have early response (two have CR), but all of them developed relapse and died.

Variable results were published dealing with ASCT in EATL, and these are summarized in Table 3. Our results are consistent with that of others [1,14,16]. Encouraging results came from a recent report by Bishton and Haynes [18] that describes the treatment of six patients with ASCT after receiving two cycles of ifosphamide, etoposide and epirubicin (IVE), followed by two cycles of high-dose methotrexate (3 g/m$^2$) with folinic acid rescue and carmustine, etoposide, cytarabine and melphalan (BEAM). Four patients remain alive in CR at 1.83–4.32 years; two had relapse. However, it is important to recognise that Bishton et al. treated patients at an early stage of disease (40–59 years old); five of them had stage I and one patient had stage II at inclusion, in contrast to our patients who were both older (60–69 years) and had advanced stage of disease (stage IV). Another important difference was the use of more aggressive chemotherapy by Bishton et al. The additive value of gut sterilisation before ASCT is difficult to interpret because of the lack of comparative data.

Cytoreductive therapy, using chemotherapy and partial small bowel resection, seems logical. We have recognized that the patients' condition improves before chemotherapy and also prevents the occurrence of complications as perforations, fistulas and bleeding.

Intervention at an earlier stage in the evolvement of lymphoma at the pre-malignant phase (RCD with high percentage of aberrant T cells) could theoretically prevent or delay the development of malignant phase. Recently, we reported on our experience in treating RCD patients with high-dose chemotherapy followed by ASCT, and the results thus far are promising in five of the seven transplanted patients [24]. A possible explanation might be that transplantation may eliminate the aberrant T-cell clone in RCD patients; this is in contrast to patients with EATL, who already have developed a neoplastic clone.

It is a well known that T-cell malignancies do not respond adequately to conventional chemotherapeutic treatment [25,26]. The introduction of monoclonal antibodies for the treatment of cancer may change the outlook for patients with T-cell malignancies [27]. Recent studies with single-agent alemtuzumab, an anti-CD52 monoclonal antibody, have shown some improvement of response rates and survival

Table 3
Summary of the earlier reports on ASCT in EATL

| Author/reference | No. EATL patients | No. received ASCT | High-dose chemotherapy | Pre-conditioning | Overall survival | Comments |
|---|---|---|---|---|---|---|
| Gale et al. [1] | 31 | 1 | PEACE-BOM | BEAM | CR 64 months | Overwhelming sepsis after ASCT |
| Okuda et al. [14] | 1 | 1 | Eight-cycle CHOP. Al relapse ESHAP (etoposide, methyl-prednisolone, cytarabine and cisplatin) | MCVC (ranimustine carboplatin, etoposide and cyclophosphamide) | 8 months | Died after developing relapse (intestine and CNS) |
| Rongey et al. [15] | 1 | 1 | Four cycles (cyclophosphamide, doxorubicine and etoposide) and then three-cycle CHOP | BEAM | CR 18 months | EATL after having coeliac disease and follows gluten free diet irregularly |
| Jantunen et al. [16] | 5 | 5 | CHOP | BEAC 3 patients/BEAM 2 patients | Median survival 2 months (0–14 months) | Four treated initially with partial small intestine resection |
| Blystad et al. [17] | 2 (total 40 NHL*) | 2 | Specific details over these two patients are not available | – | – | – |
| Bishton and Haynes [18] | 6 | 6 | Two cycles of ifosphamide, etoposide and epirubicin (IVE), followed by two cycles of methotrexate (3 g/m$^2$) + folinic acid rescue | BEAM | Four in CR at 1.83–4.32 years | Two had relapse. Relatively younger patients and treated at an early stage of disease |

* NHL = non-Hodgkin's lymphoma.

in patients with T-cell prolymphocytic leukaemia and cutaneous T-cell lymphoma [28]. Preliminary data also suggest that alemtuzumab may have activity in patients with heavily pre-treated peripheral T-cell lymphoma who are refractory to conventional chemotherapy [29]. Pre-clinical studies with mice bearing human adult T-cell leukaemia/lymphoma cells suggest that alemtuzumab may have a role in this setting [30]. Therefore, treatment of EATL with alemtuzumab in combination with chemotherapy should be explored.

Furthermore, it has been shown that patient who undergo allogeneic SCT for NHL, both indolent and high-grade types, have lower relapse rates than those who undergo autologous SCT [31–33].

It seems that current chemotherapy and high-dose conditioning regimens followed by ASCT do not improve the survival in this type of aggressive lymphoma. Relapse regularly occurs within weeks to months after ASCT. Therefore, instituting therapy at an earlier stage, the development of more effective treatments including anti-CD52 agents, better pre-conditioning regimens and possibly use of T-cell-depleted grafts or allogeneic stem cell transplantation with or without primary central nervous system prophylaxis are urgently required to improve the prospects of these patients.

**Practice points**

- Enteropathy associated T-cell lymphoma can be $CD3^+$ $CD8^-$ $CD30^+$ large cell lymphomas, $CD3^+$ $CD8^+$ $CD30^-$ small cell lymphomas or γδ-lymphomas.
- Chemotherapy in EATL can be complicated by small bowel perforation, gastrointestinal bleeding and development of enterocolic fistulae.
- Cytoreductive therapy, using chemotherapy and partial small bowel resection, seems logical.
- It seems that current chemotherapy and high dose conditioning regimens followed by ASCT do not improve the survival in this type of aggressive lymphoma.

**Research agenda**

- Intervention at an earlier stage in the evolvement of lymphoma at the premalignant phase (RCD with high percentage of aberrant T cells) could theoretically prevent or delay the development of the malignant phase.
- Instituting therapy at an earlier stage, the development of more effective treatments including antiCD52 agents, better preconditioning regimens and possibly the use of T cells-depleted grafts or allogeneic stem cell transplantation with or without primary central nervous system prophylaxis are urgently required to improve the prospects of these patients.

## Conflicts of interest statement

None declared.

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## LETTER TO THE EDITOR

# Disappointing outcome of allogeneic hematopoietic SCT in two EATL patients

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Enteropathy-associated T-cell lymphoma (EATL) is associated with refractory celiac disease (RCD).[1,2] Approximately, 50% of patients with CD are diagnosed in adulthood; of them, 2–5% develop RCD. RCD patients comprise two groups: one with immunophenotypically aberrant intraepithelial lymphocytes (RCDII) and one without these cells (RCDI), the cutoff being 20%.[2] Aberrant intraepithelial lymphocytes are characterized by the lack of surface expression of CD3 and often also CD8. Overall, 60–80% of RCDII patients develop EATL within 5 years.[3]

Enteropathy-associated T-cell lymphoma is almost invariably lethal, although high-dose chemotherapy followed by Auto-SCT might improve survival.[3] Experience with myeloablative or reduced-intensity conditioned (RIC) Allo-SCT in T-cell non-Hodgkin's lymphoma is limited, but promising.[4] We report the first two celiac patients with EATL in whom we performed Allo-RIC-SCT from an HLA-identical sibling donor.

### Case 1

A 66-year-old man, recently diagnosed with CD, presented with weight loss and night sweats. Abdominal CT showed increased jejunal wall thickness and mesenterial lymphadenopathy, suggestive of EATL.[5] Double balloon enteroscopy revealed ulcerative jejunitis and stenosis due to multiple masses. A diagnosis of EATL was confirmed by biopsies. BM investigation revealed 15% aberrant T cells (CD3+, CD7−).[2] He underwent a resection of the involved small bowel segment, with primary anastomosis. After surgery there were no signs of short-bowel syndrome and a strict gluten-free diet was started. Post-surgical FDG-PET evaluation showed multiple lymph nodes suspicious of residual EATL. Treatment with six cycles of CHOP chemotherapy was initiated and subsequent FDG-PET showed CR.

HLA typing identified two siblings as fully matched donors. One of them was diagnosed with CD during pre-transplant screening. The other sibling, with a negative celiac serotype, was therefore selected. At 6 weeks after the last chemotherapy, Allo-RIC-SCT was performed following conditioning with fludarabine (25 mg/m$^2$) and CY (500 mg/m$^2$) (flu-cy) for 5 consecutive days. A total of $4.8 \times 10^6$ CD34+ cells/kg were infused after conditioning. Donor chimerism after 1 and 2 months was 83 and 90%, respectively.

At 2 months after Allo-SCT the patient developed a relapse of EATL. Rapid deterioration precluded further treatment, and the patient died 3 months after Allo-SCT.

### Case 2

A 61-year-old man was admitted with perforation of the jejunum. He underwent partial surgical resection of the jejunum. Pathological examination revealed perforation due to EATL (Marsh IIIA). BM investigation revealed 0.01% aberrant T cells.

After surgery there were no signs of short-bowel syndrome and a strict gluten-free diet was initiated. Post-surgical FDG-PET evaluation showed intense uptake in the left upper part of the abdomen. There were no other signs of EATL. After six cycles of CHOP chemotherapy FDG-PET showed CR.

The patient was subsequently transplanted from an HLA compatible matched sibling 2 months after finishing chemotherapy. A total of $8.3 \times 10^6$ CD34+ cells/kg were infused after conditioning. At 1 month after transplantation, donor chimerism was 23% in peripheral blood and 19% in BM.

At 6 weeks after transplantation, the patient developed non-specific abdominal complaints and a rise in lactate dehydrogenase. As relapse was suspected, immunosuppressive medication was discontinued. After 2 weeks, relapse of EATL was confirmed. His condition deteriorated quickly and 9 weeks after Allo-SCT the patient died.

To date, treatment of EATL is disappointing. Recently, several studies with high-dose chemotherapy and Auto-SCT have been published. These regimens showed improving results, but only in selected patients. Bishton and Haynes[3] illustrated the beneficial effect of high-dose chemotherapy in six patients. In total, 4 patients are still alive after 2–4 years follow-up. Other reports, however, showed less favorable results.[6–8] New strategies are therefore urgently needed.

Theoretically, Allo-SCT might be a treatment option for patients diagnosed with EATL. In a prospective, multicenter phase II trial, a total of 170 elderly ($\geqslant$45 years) patients with relapsed or refractory lymphomas of all types (including 14% aggressive T-cell non-Hodgkin's lymphoma) received Allo-RIC-SCT from HLA-identical sibling donors. This procedure was found to be feasible and effective. A trend toward a higher relapse rate was observed in patients with aggressive lymphoma in PR or with refractory disease before transplantation. Withdrawal of CYA contributed to the clinical response. Median time to progression was 6 months.[9] In an earlier study, a poor outcome of Allo-RIC-SCT in patients with chemoresistant and

high-grade lymphomas (including T-cell non-Hodgkin's lymphoma) was reported.[10] Although this retrospective study failed to show an effect of intensity of conditioning, it was hypothesized that these patients benefited less from Allo-SCT, as the beneficial effect of GVL takes a few months to develop. Donor lymphocyte infusion was successful, illustrating a GVL effect.[10]

We decided to offer Allo-RIC-SCT to chemosensitive patients in CR using our flu-cy conditioning scheme. As HLA DQ2 and DQ8 are associated with CD, the donor search needed special consideration to exclude donors with CD by serological screening.

The two cases presented are the first EATL patients treated with Allo-RIC-SCT. The experimental setting led to the selection of HLA-identical siblings only. Although both patients were chemosensitive and in CR before transplantation, they developed relapse of EATL within a few weeks after transplantation. As a GVL effect could not have occurred, the conditioning probably was not sufficiently potent to buy time.

Possibly more intensive consolidation, for instance the Bishton schedule, is necessary to improve current results in this highly aggressive lymphoma.[3] Moreover, it may be that introducing GVL in an earlier stage, established by more rapid exclusion of immunosuppressive medication, might improve results.

In conclusion, this first report of Allo-RIC-SCT in patients with EATL and CD illustrates its feasibility but also disappointing results in two patients in CR in excellent condition pre-transplant. It is possible that more intensive consolidation is needed to improve results.

## Conflict of interest

The authors declare no conflict of interest.

JC Regelink[1], GJ Tack[2], PC Huijgens[1], CJJ Mulder[2], JJWM Janssen[1] and O Visser[1]
[1]*Department of Haematology, VU University Medical Center, Amsterdam, The Netherlands and*
[2]*Department of Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands*
*E-mail: j.regelink@vumc.nl*

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